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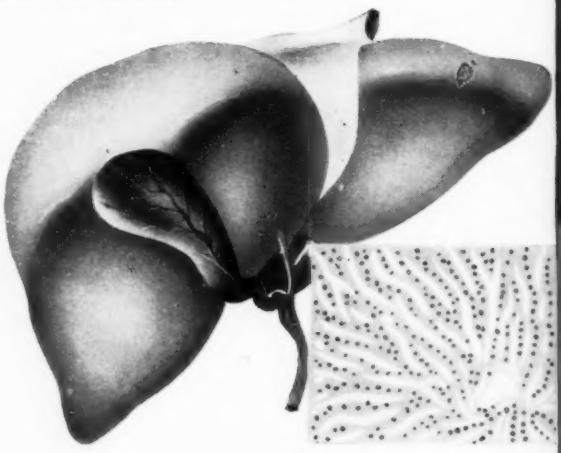

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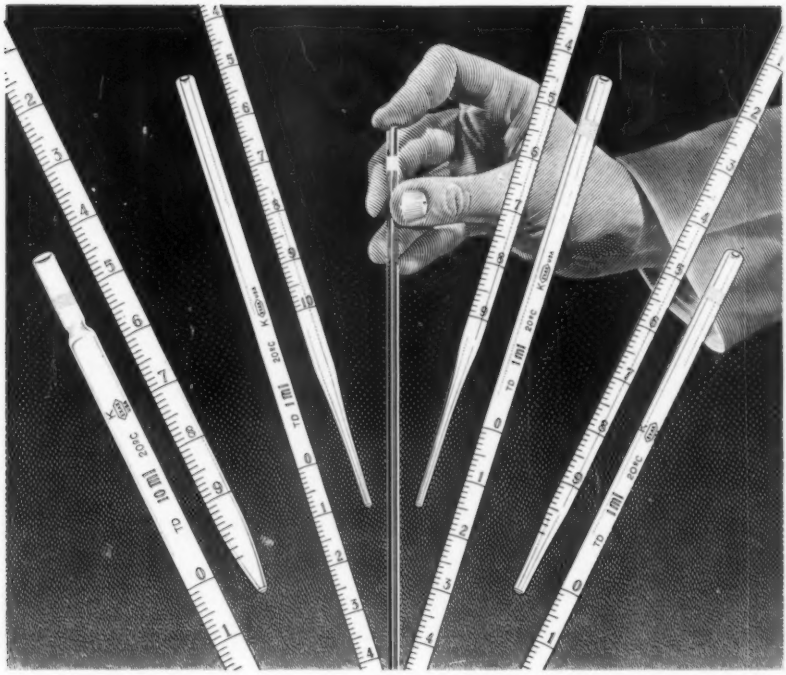

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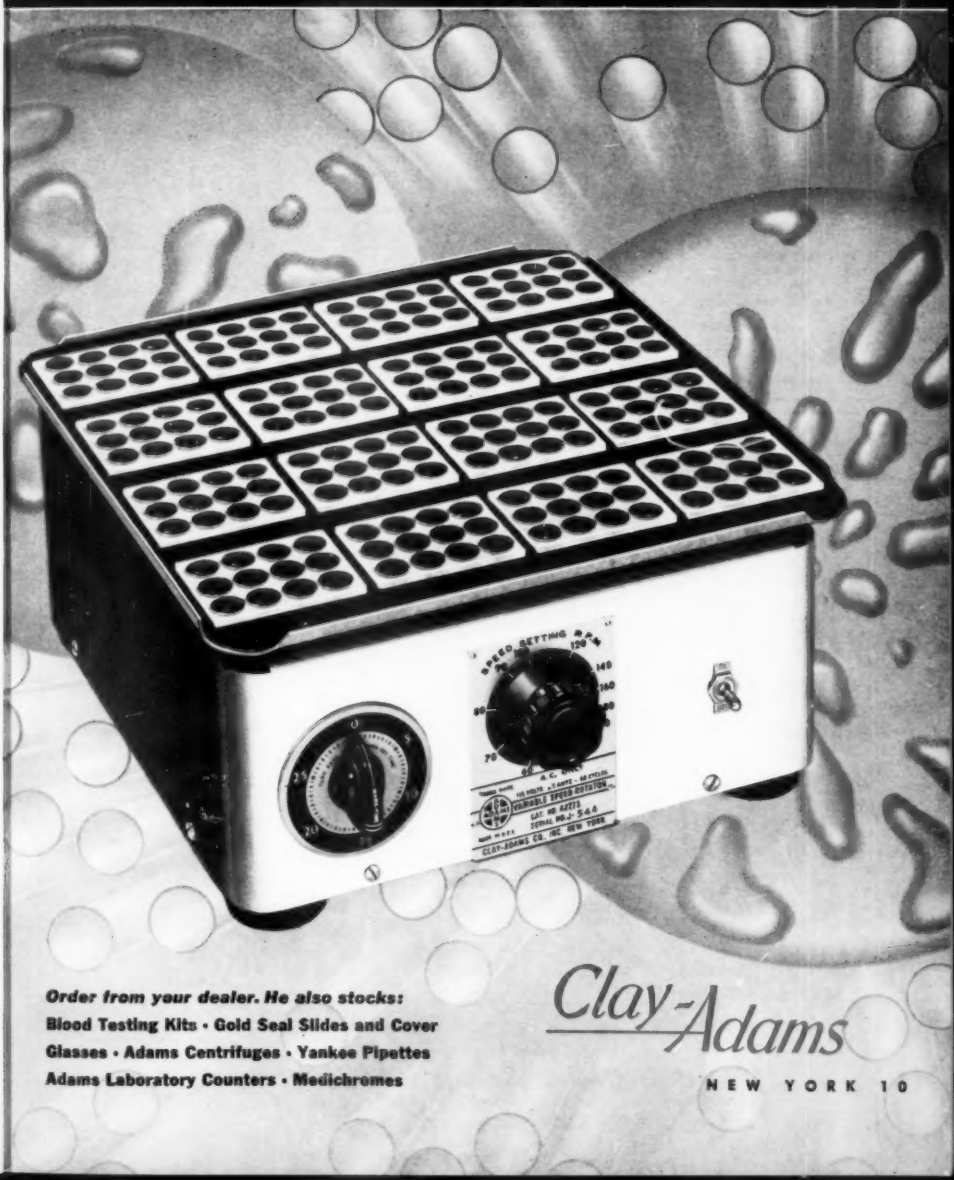

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[1] Nelson, R. A., Jr.: Science, 118: 733, 1953.
[2] Portnoy, J., and Magnuson, H. J.: J. Immunol., 75: 348, 1955.

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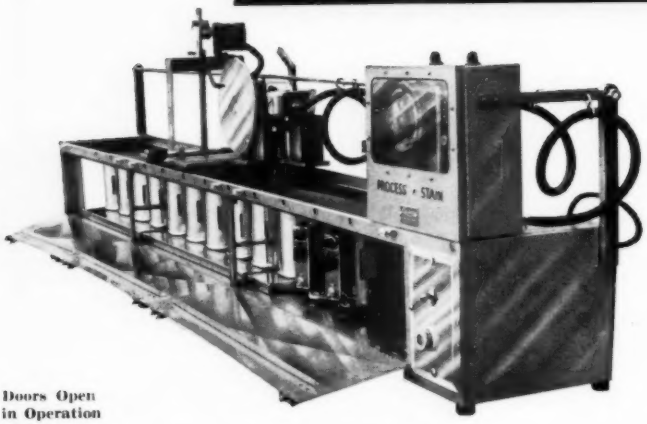

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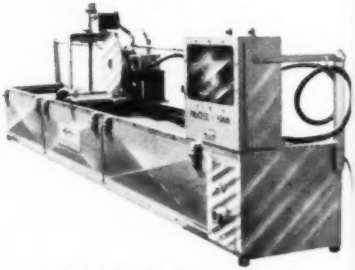

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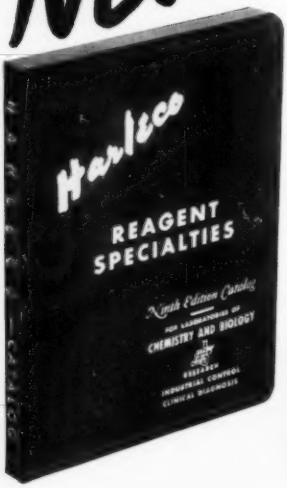

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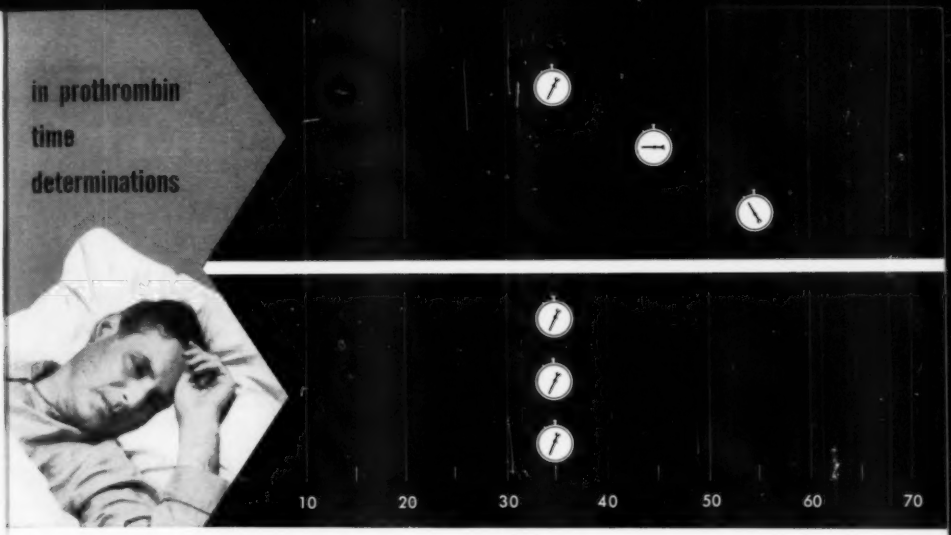

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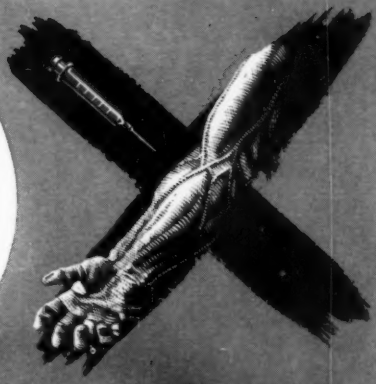

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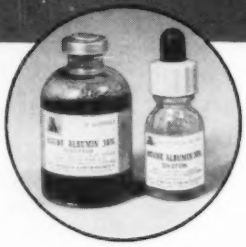

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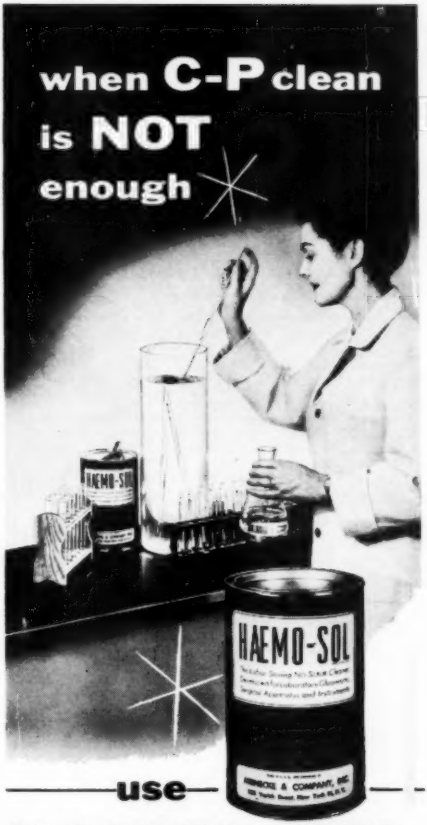

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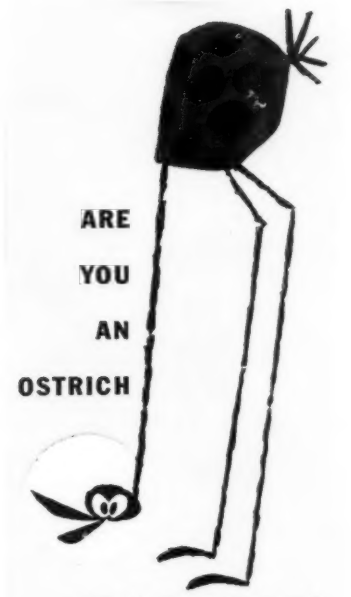

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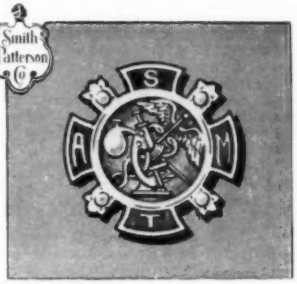

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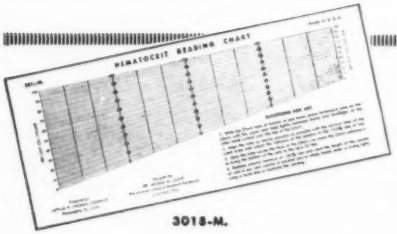

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AMPEROMETRIC TITRATION OF CHLORIDE IN TISSUE, SERUM, URINE, AND FECES*

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University of Minnesota, Minneapolis, Minn.

For studies on the electrolytes of heart muscle it was desired to use a chloride method which was more micro and with a better end point than the Volhard titration that is so often used. The accuracy of the Volhard titration as used in the Van Slyke method for tissue chloride has been questioned by Williams, Seibert, and Huggins.[1] They found that liver chloride values were 26% lower by the Van Slyke method than by gravimetric method. This they attributed to the poor end point obtained in the presence of chromogenic material in the liver. Acid extracts of heart tissue are also colored and it is presumed that this would cause the same end point difficulty. The amperometric titration eliminates this end point trouble and has the additional advantage of being well suited for dilute solutions. It proved so satisfactory for tissue that it was later applied to serum, urine, and feces.

Several papers have been published on the amperometric titration of chloride. The more recent ones are those of Kolthoff and Kuroda[2] describing the titration of low concentrations and of Carr[3] showing that an earlier method of Laitinen, Jennings and Parks[4] is adaptable for the determination of plasma and urine chloride. It is Carr's method which has been modified for this work by reducing the volume of solution to be titrated.

The theory of amperometric titrations has been covered in detail by Kolthoff and Lingane[5] and will not be discussed here. The text of Willard, Merrit, and Dean[6] is helpful for both theory and practice.

* Registry Award, read before ASMT, New Orleans, La., June, 1955. Tied for Second Award, 1955, Scientific Products Foundation, Biochemistry Division.

METHOD

Reagents

Silver nitrate solution, 0.01 N. Dissolve 1.6989 gm. $AgNO_3$ in distilled water and dilute to one liter. Store in a brown bottle.

Nitric acid, concentrated, analytical grade.

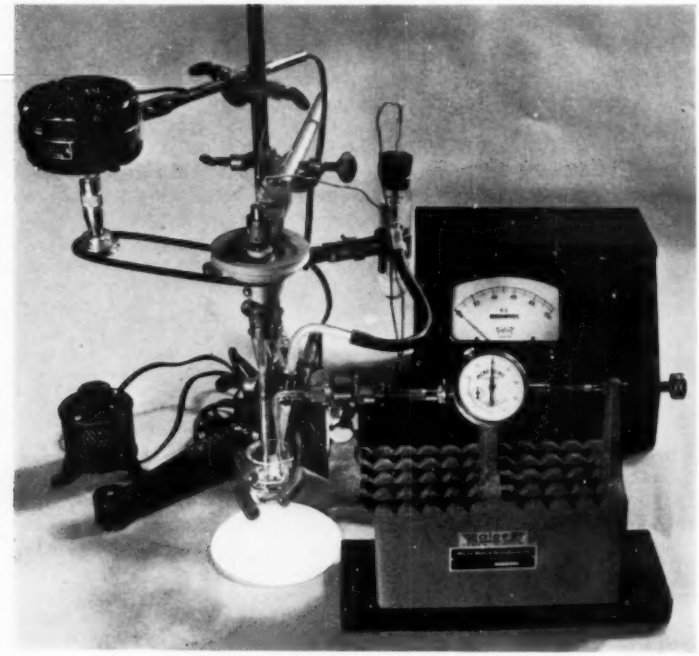
Nitric acid, 1.5 N. Dilute 93.8 ml. of concentrated nitric acid to one liter.

Gelatin, 1%. Dissolve 1 gm. in 100 ml. of distilled water with the aid of heat. Store in the refrigerator to prevent the growth of bacteria.

Acetone, reagent grade.

Apparatus

The apparatus consists of a saturated calomel electrode, a platinum micro-electrode, a stirring motor, a micrometer, and a ring stand with clamps as shown in figure 1.



There are numerous forms of the calomel electrode, but a suitable one can be prepared according to Kolthoff and Furman.[7] Electrical connection between it and the solution being titrated

is made by a salt bridge consisting of a glass tube filled with a solution of 30 gm. potassium nitrate, 3 gm. agar, and 100 ml. water. When not in use, the tip of the bridge is immersed in a solution of potassium nitrate.

A piece of 18 or 20 gauge platinum wire about 10 mm. long is sealed into the end of a 7 mm. OD soft glass tube to form the rotating microelectrode as follows: The end of the tube is rotated in a flame until the hole remaining is just large enough to admit the wire. Then the wire is inserted leaving about 5 mm. projecting and the heating continued until the opening is sealed. While the glass is still soft, the wire is pulled slightly to give better adhesion between the wire and glass. At the same time the end of the rod is given a slight bend to increase the stirring action. The seal is tested by dipping the electrode into water and blowing. A few centimeters of mercury are placed in the tube so that electrical contact is made between the platinum wire and the stationary copper wire of the external circuit.

A stirring motor with a hollow shaft to admit the rotating electrode is recommended such as that manufactured by Eberbach of Ann Arbor, Michigan. A motor of this type greatly simplifies the electrical connection between electrode and micrometer. Willard et al[6] describe an electrode for use with an ordinary stirrer.

An inexpensive micrometer with a 0-100 μa scale graduated in 2 μa divisions is used. A Triplet model 420 meter was used in this work. The positive terminal of the meter is connected to the wire from the rotating platinum electrode and the negative to the calomel cell.

A micro burette with an offset tip, a capacity of 1-2 ml., and an accuracy of $\pm$ 0.001 ml. is required. A syringe type burette* incorporating a 2 ml. hypodermic syringe and a dial micrometer reading in inches was used here.

Tissue

Homogenize 1 gm. of minced tissue in 14 ml. of distilled water using a Potter-Elvehjem type homogenizer. Add 15 ml. of 1.5 N nitric acid to the homogenate and allow to stand 30 min. (A 5 ml. aliquot of the water homogenate can be diluted 1 to 2 with acid if other determinations require a water homogenate or extract. The acid extract can also be used for sodium and potassium determinations). Centrifuge and titrate as follows:

Pipette 4 ml. of supernatant extract into a 20 ml. beaker, add 0.4 ml. of 1% gelatin, 0.5 ml. of concentrated nitric acid, and 8 ml. of acetone. Clamp the beaker in the apparatus, start the electrode rotating, and record the residual current after it has reached equilibrium. The current will be high at first, especially with a new electrode, then rapidly drops to about 1-2 μa. Add 0.01

* Micro-metric Instrument Co., Cleveland, Ohio.

N silver nitrate from a micro burette until a sudden rise in current occurs. Then add the silver nitrate in increments, recording current and volume with each addition until five or six points are obtained. Plot the values of current (ordinate) against volume of silver nitrate (abscissa) as illustrated in figure 2. The intersection of two straight lines locates the end point.

Determine the titer of the silver nitrate by titrating in a similar way 4 ml. of a 2 meq./l. standard sodium chloride solution.

Serum (or plasma)

Titrate 4 ml. of a 1 to 50 dilution of serum or 0.02 to 0.1 ml. of serum delivered into 4 ml. of water in a manner similar to the titration of muscle extract except that 0.2 ml. of 1% gelatin and 7 ml. of acetone are added.

Urine

Titrate 4 ml. of a 1 to 50 dilution or 0.1 ml. delivered into 4 ml. of water exactly as for tissue.

Feces

Homogenize a weighed portion in water to give a 1 to 50 dilution. Add 1 volume of 1.5 N nitric acid to an aliquot of the homogenate, centrifuge, and titrate 4 ml. of the supernatant extract in the same way as for tissue. If a precipitate forms on the addition of acetone, transfer to a centrifuge tube, stopper, and centrifuge. Titrate 10 ml. of this supernatant directly.

Results

Varying amounts of standard sodium chloride solution ranging from 2 to 8 μeq were titrated to test the linearity of the titration at different concentrations. The amount of silver nitrate required at the equivalence point was found to be proportional to the amount of chloride present over this range. This is shown graphically in figure 3.

The results of single determinations on duplicate extracts of dog cardiac muscle are shown in table 1. Good precision was obtained.

In table 2 are shown the results of duplicate determinations on 18 sera run over a period of one month by both the Schales and Schales[8] method and the amperometric method described here on 0.1 ml. of serum. They agree within 2%. The precision is the same for both, each having a standard deviation between duplicates of $\pm$ 0.74.

Single determinations on urine by the modified Volhard and Harvey[9] method are compared to those by amperometric titration in table 3. Agreement is not as good as for plasma, and this may be due to the less precise end point in the Volhard titration.

Chloride values on rat feces were found to be very low and although lower than the sodium values they were parallel to them.

Good recoveries were obtained on serum, urine, and heart

TABLE 1

Comparison of chloride values on duplicate homogenates of dog heart muscle by amperometric titration.

Dog No. X Source	Meq./kg. Wet Wt.	Difference Between Duplicates
8 Septum	36.6 36.5	0.1
10 Right Ventricle	27.1 26.8	0.3
10 Left Ventricle	26.0 24.6	1.4
*11 Right Ventricle	42.4 43.7	1.3
*11 Left Ventricle	42.3 42.2	0.1
*12 Right Ventricle	33.6 33.4	0.2
*12 Left Ventricle	34.8 34.6	0.2
14 Right Ventricle	24.7 25.2	0.5
14 Left Ventricle	26.5 24.2	2.3
*27 Right Ventricle	28.3 27.8	0.5
*30 Right Ventricle	26.6 26.5	0.1
31 Left Ventricle	25.0 25.7	0.7
32 Right Ventricle	25.3 25.1	0.2
35 Left Ventricle	25.7 26.4	0.7
*36 Left Ventricle	27.7 27.7	0.0
		Average 0.57 SD ± 0.51 SD in % of mean. 1.7

* Experimental animal.

tissue as shown in table 4.

DISCUSSION

Gelatin is added to the solution to coat the colloidal particles of silver chloride in order to prevent them from depolarizing the cathode.[4, 2, 3]

The addition of nitric acid prevents the formation of interfering insoluble silver salts such as phosphates, oxalates, and proteinates. A nitric acid extract of tissue was chosen as a suitable sample for chlorides following the work of Hastings and Lowry[10] which showed agreement between a Volhard titration of a nitric acid extract and an open Carius digestion. Robertson and Pey-

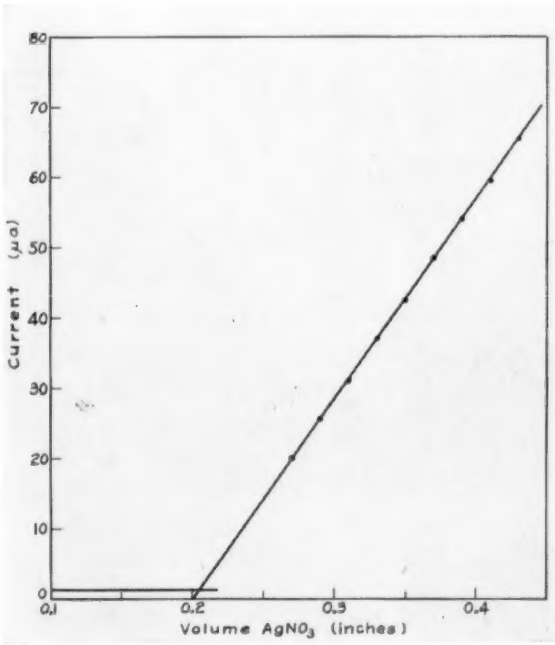


Figure 2. Typical graph of titration data.

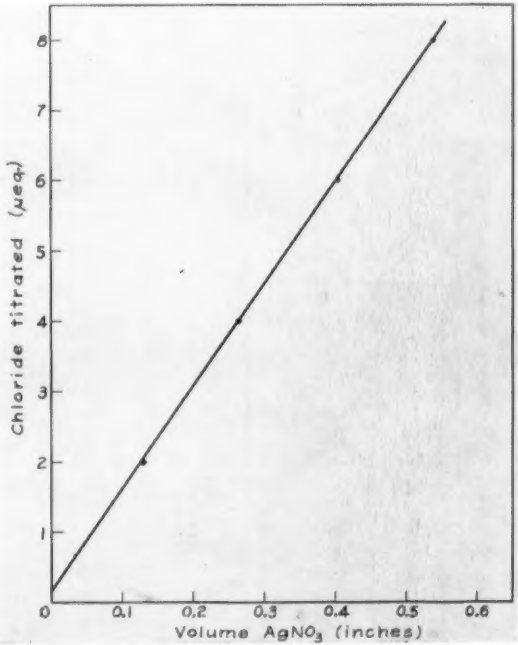


Figure 3. Graph showing linearity between volume of silver nitrate required and the amount of standard chloride titrated.

TABLE 2

Comparison of serum chloride values obtained by different methods on 18 sera over a period of one month. Means and standard deviations computed using all 18 values.

Schales & Schales Meq./l.		Amperometric Meq./l.		Difference
102 104	103 Avge.	106 105	105.5 Avge.	+2.5
92 91	91.5	91 91	91	—0.5
112 111	111.5	114 113	113.5	+2.0
87 88	87.5	88 88	88	+0.5
103 102	102.5	97 97	97	—5.5
Mean N = 18	103.0		104.1	2.06
SD Between Duplicates.....	±0.74		±0.74	
SD as % of Mean....	0.72		0.71	

TABLE 3

Comparison of urine chloride values determined by different methods. Only five of the ten specimens studied are shown.

Volhard-Harvey Meq./l.	Amperometric Meq./l.	Difference
134	140	6
85	83	2
249	256	7
75	74	1
102	102	0
Mean N = 10..... 146.7	151.2	5.1
SD Between Duplicates.. ±1.5	±1.9	
SD as % of Mean..... 1.0	1.3	

TABLE 4

Amperometric Titration of known amounts of chloride added to plasma, urine, and heart muscle.

SAMPLE	Chloride Meq./l.	Chloride Added Meq./l.	Total Chloride Amp. Titration	Chloride Recovered Meq./l.	Percent Recovery
Plasma.....	103.7	33.3	137.5	33.8	101.5
Urine.....	64.3	33.3	96.8	32.5	97.6
Heart.....	36.6	20.0	55.7	19.1	95.5

ser[11] have also used a nitric acid extract of minced heart muscle.

The solubility of silver chloride is depressed by the addition of acetone,[4] allowing sharp end points to be obtained on low concentrations of chloride. Although Laitinen et al[4] recommend up to 75% acetone, it was found necessary to use less for samples containing protein such as serum to prevent the precipitation of protein. Any flocculent precipitate interferes by coating the platinum wire. As long as the proteins remain in solution or in a colloidal state, they do not interfere but serve the same function as gelatin. For this reason less gelatin is added for the titration of serum.

Although this work was carried out at room temperature, it is preferable to keep the temperature of the solution being titrated as low as possible, especially with lower concentrations of chloride.[2] The sharpness of the end point depends on the solubility of silver chloride which increases rapidly with rising temperature.

The electrode should be driven at constant speed to prevent fluctuations in the diffusion current. Although it has been stated that the speed should be 600 r.p.m., a greater speed was found to be satisfactory.

Cleaning of the electrode between samples appears to be unnecessary, but occasionally it may be cleaned by rotating a few minutes in concentrated nitric acid until a bright platinum surface is obtained. After cleaning, a very high residual current will be noted, but this will drop slowly to a low value after several minutes rotation in a titration mixture.

The method is trouble-free except for the salt-agar bridge. Deterioration of the bridge is usually indicated by poor duplicates and rising values on a standard solution. Instead of using a bridge until poor results were obtained, it was found best to replace the bridge at periodic intervals of about two weeks. Replacement is quick and simple.

SUMMARY

An argentometric, amperometric titration of chloride in muscle, serum, urine and feces is described. Good agreement is demonstrated on serum and urine between the method described and the Schales and Schales and Volhard-Harvey methods respectively. Single determinations can be made on a minimum of 2 μeq. of chloride (0.02 ml. of serum or plasma, 0.02 to 0.1 ml. of urine, or ca 165 mg. tissue). Recoveries on plasma, urine, and tissue of 101.5, 97.6, and 95.5% respectively were obtained. Precision expressed as percent standard deviation of the mean is 1.7 for tissue, 0.71 for serum, and 1.3 for urine.

ACKNOWLEDGMENT

The author wishes to thank Miss Esther Freier, Instructor in Chemistry, University of Minnesota Hospitals, for her helpful criticism.

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Our gracious hosts have many wonderful things planned for those attending this conference. As their guests we can expect various deviations from our usual routine; however, our business meeting will be on Wednesday and Friday. Should you consider presenting resolutions or other items of business for the Advisory Council or House of Delegates please send a copy to the President before June. Your wonderful cooperation in returning the questionnaires reflects your interest in the society, and we trust you will continue to make your desires known.

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"TRACER LEVEL" PROCEDURES

For want of a better term we call this the use of isotopes at the "tracer" level. It is appropriate in that it reminds us that we are working with very small quantities, both as far as weight and activity are concerned. So small are the amounts used, that with reasonable judgment and precautions, there is no reason why any worker ever need receive more than a minute fraction of the radiation per year that he or she would get from a dental X-ray. In fact, there is no evidence that anyone working in a hospital diagnostic laboratory has ever been harmed in any way by radiation from radioisotopes.

For work of this sort the laboratory usually does not require much, if any, modification. (Illustration I.) The installation of stainless steel trays on the desks' top to catch any spills is desirable. (Illustration II.) So are (1) a cubicle made of ¼" lead sheet in which contaminated glassware may be kept segregated, (2) a small storage area (such as a refrigerator) some 10-15 feet away for the storage of supplies and active samples, and (3) a portable laboratory monitor to assist in "good housekeeping." No other shielding is necessary and unused activity is simply put into the sewage system at some point where it will not raise the radiation "background." Unless many samples are to be evaporated, a hood, though convenient, is not essential.

Since isotopes obviously do not harm us (and may be handled

* Read before ASMT, June, 1955, New Orleans, La.

Fig. I

Fig. II

TABLE 1
Radioisotopes Are Used to Obtain Clinically Valuable Information

	Dosage Range
1. Thyroid uptake and excretion studies (I-131) (BMR) (Radiocaps)	2–50 uc.
2. Protein Bound Iodine and conversion ratios (I-131) (Tracervial)	35–75 uc.
3. Thyroid scanning and mapping (I-131)	200–300 uc.
4. Total Blood Volumes—Plasma Volumes (RISA—Rachromate)	5–50 uc.
5. Red cell Mass and Survival Times (Rachromate)	75–200 uc.
6. Blood loss by bleeding (Rachromate)	250–300 uc.
7. Liver Function (I-131 Rose Bengal)	5–15 uc.
8. Pernicious Anemia (Co-60 Cyanocobalamine)	0.5 uc.
9. Iron Turnover Rates (Fe-59 Ferrous Citrate)	10–30 uc.
10. Total Body Water (D_2O), Tritium Oxide, or 4-Iodoantipyrene	50–100 gm. (D_2O)
11. S-35 as Na_2SO_4	.....
12. Circulation Times—Cardiac Outputs (RISA — — Na-22)	10–25 uc.
13. Liver Circulation ($CrPO_4$)	
14. Thiocyanate, Sodium and Potassium "spaces", etc.	
15. Presence and location of Brain Tumors (RISA)	100–500 uc.
16. Eye and Skin tumor diagnosis (P-32)	500 uc.

Radioisotopes Are Widely Used As Sources of Either Beta or Gamma Rays for Therapeutic Purposes

1. Polycythemia and certain lukemias	P-32, $CrPO_4$	2–12 mc.
2. Hyperthyroidism and Cardiac Dysfunction	I-131	3–15 mc.
3. Some Thyroid tumors	I-131	10–150 mc.
4. Pleural and Peritoneal effusions	Au-198, $CrPO_4$, Y-90	50–150 mc.
5. Inoperable tumor masses (Prostate)	$CrPO_4$, Au-198, Y-90	50–150 mc.
6. Interstitial therapy (gamma)	Cobalt Needles Cobalt Applicators Cobalt in Nylon	50–75 mc.

in most laboratories), let us see how they can help us in our various laboratory procedures. Table 1 outlines some of the diagnostic tests to which they have been successfully applied, the substances used in each, and the general range of activities employed. To help understand this, let us look also at Table 2 which lists the principal isotopes used in medicine, and certain properties we must learn to utilize. By half-life we simply mean the time required for one-half of the activity to have disappeared. There is obviously a practical lower limit to this half-life, since the isotope must be available when needed. The upper limit (usually about 30-60 days) is set by the fact that we do not wish to unnecessarily subject the patient's body to continuing radiation once the test or experiment is completed. Here a second factor usually operates in our favor. Compounds are usually metabolized or excreted fairly rapidly, so that many isotopes and tagged compounds have a far shorter "biological half-life" than would be true if we depended on radioactive disintegration alone.

RADIATION AND ITS MEASUREMENT

You will note next that at the time each atom disintegrates, it gives off either one or both of two types of radiation, each with characteristic properties. The gamma rays of Table 2, column 5,

TABLE 2
Isotopes Currently Being Used in Medical Diagnosis, Therapy and Research

Isotope	Half Life	Days Effective Half Life	Beta MEV	Gamma MEV
K-42	12.4 hr.	0.51	2.04 3.58	1.5
Cu-64	12.9 hr.	0.53	0.6 b	1.3
Na-24	14.8 hr.	0.61	1.39	1.38 2.75
Br-82	34.0 hr.	....	0.465	1.35 etc.
Y-90	2.5 d.	....	2.18	None
Au-198	2.7 d.	2.6	0.97 0.6	0.41
I-131	8.0 d.	7.7	0.60 0.31	0.638 0.37
P-32	14.3 d.	14.0	1.71	None
Rb-86	19.5 d.	7.8	1.88	1.1
Cr-51	26.5 d.	22	....	0.32 (k)
Fe-59	46.0 d.	27	0.46	1.3
S-35	87.1 d.	18	0.17	None
Ca-45	152.0 d.	....	0.26	None
Zn-65	250.0 d.	8.0	0.32 b	1.1
Na-22	2.6 yr.	8.0	0.56	1.28
Co-60	5.3 yr.	8.4	0.31	1.16 1.32
H-3	12.5 yr.	19.0	0.019	None
Ra-226	1700.0 yr.	104.0	....	0.8 approx.
C-14	5700.0 yr.	180.0	0.15	None

are in effect X-rays, and are little absorbed by the tissue or fluid in which they originate. This is important because it may mean that we do not need to combust or even disintegrate a tissue to find out how much of a gamma emitting isotope or compound is contained therein. In fact, we do not need to expose or even see or feel that tissue to be able to measure the activity. For instance, we can determine quantitatively the amount of radio-iodine in the thyroid from a position a foot or more away from the patient. Since color, opacity or the presence of other substances makes no difference in gamma measurements, we avoid many tedious purification and isolation steps. Chromatography is particularly applicable to labeled compounds, and since we do not need to depend on reagents to develop colored bands or spots, but can assay the strips directly for the radioactivity at various points, these procedures, too, are markedly improved. The beta rays in column 4 of Table 2 are of little use to us in the laboratory but I would like to emphasize one unique property, i.e., they are stopped by very small (less than 1 cm.) thicknesses of tissue, giving up thereto all of their energy. Thus we have a powerful tool for tissue destruction and we can treat hyperthyroidism and certain tumor masses just as effectively, and certainly more conveniently, than by surgery. It may surprise you to know that many isotopes are produced for such therapeutic uses. This is, however, beyond the scope of our discusssion here.

SPECIFIC DIAGNOSTIC USES
(The Thyroid Uptake)

I imagine that most of you have had some experience with the BMR determination. You have seen how the values may vary

as the result of emotional stress, physical exertion and other totally extraneous factors. You know the mental reservations and the physical restraints of the test. Perhaps you have sensed the hesitancy with which many physicians accept such a single BMR determination.

The isotopic "equivalent" has few of these disadvantages. The technique commonly employed involves the oral administration of a single tightly sealed capsule (Radiocap®) containing (Illustration III) a carefully measured minute amount of (5-50 uc.) of sodium radio-iodide (I-131). In the type developed in the author's laboratory, there is no filler—the capsule is to all intents and purposes empty, having the activity tightly adsorbed on the interior wall. Even if dropped there is no breakage or contamination. This capsule can be given to the patient by the physician to be taken in his room or even at home, if this is more convenient. The subject reports back 24 hours later for one or more measurements of the per cent of the I-131 taken which is now in the thyroid. This is a quantitative measure of thyroid function, and thus of the basal metabolic rate of that individual. Such a simplified test serves to give a reasonably clear diagnosis of uncomplicated cases, but the real advantage of the isotopic method lies in the fact that there are a number of auxiliary tests which may be applied to provide additional information of clinical value. For instance the *rate* at which the thyroid accumulates the I-131 is quite characteristic for certain abnormalities. Hence it is becoming customary to make measurements not only at 24 hours, but also at 6 or 8 hours as well, enabling a rough "clearance curve" to be constructed. One may measure the amount excreted in the urine to check on the accuracy of ones technique and to indicate the possibility of thyroxin producing areas outside the true thyroid.

Many of you may have tried your hand at the PBI or protein bound iodine (chemical) and a very few of you may have succeeded in getting worthwhile results. Certainly the technique is not widely applicable or generally reliable at the laboratory level. Again isotopes provide a simple alternative. At, let us say, 24 hours, a small amount of the administered (I-131) iodide remains in the plasma, and there is also present there, bound to a protein, most of the I-131 thyroxin produced. By precipitating the protein plus thyroxin with trichloracetic acid and measuring the activity in the precipitate and the supernatant (preferably in a well-type scintillation counter) (Illustration IV), we have a measure of the rate of thyroxin production as a function of plasma iodide content, which is a way of measuring this aspect of thyroid activity. To date clinical correlation has not been as good as with the simpler uptake studies of the last paragraph.

Fig. III

Fig. IV

It has been arbitrarily observed that in the hypothyroid the salivary I-131 is usually elevated, and certain laboratories are employing this as a confirmatory test. If the I-131 is given intravenuously, the entire uptake (or more technically, the clearance rate) may be evaluated in 35-45 minutes. We may scan areas around the neck and chest and pick up any areas of extra thyroidal activity.

Again let me repeat that it is this versatility and the variety of dynamic information which the I-131 procedures supply, that make it clinically so much preferred to the BMR.

Fig. V

INSTRUMENTATION

What sort of instrumentation is required? Fortunately as the result of some 10 years of intensive testing and use, self-contained units just as trouble free and efficient as other laboratory electronic instrumentation are available. In general the heart of the unit is a "scintillation detector," a device which picks up each gamma ray and converts it to an electrical impulse which is fed to a recording device controlled by an accurate timer. For instance in the thyroid test this scintillation detector is mounted on a flexible arm (Illustration V) so that it may be accurately positioned with respect to the thyroid area of the patient's neck.

The counter is set to run for two minutes (or any other suitable time) and the number of counts recorded is noted. A second capsule containing the exact amount of activity as that given the patient, and contained in a suitable plastic Phantocube to simulate the patient's neck is placed at this same distance, and counted for the same length of time (Illustration VI). Dividing the patient's count by the count of the standard gives the "thyroid uptake" in per cent. In general the normal range is from 15-40%. The hypothyroid will usually show less than 10% uptake and the hyperthyroid 45-75%. The scope of this presentation does not permit a discussion of the interpretation of such data which is solely in the province of the trained clinician.

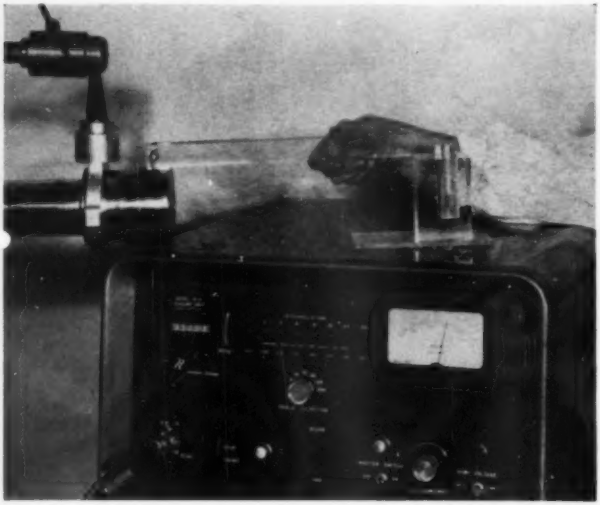


Fig. VI

BLOOD AND PLASMA VOLUME DETERMINATIONS

Another common laboratory procedure in which the use of isotopes confers many advantages is in the determination of blood volumes. For this purpose there has been developed a radioiodinated serum albumin (RISA®) which, given intravenously, mixes with the ordinary plasma. As will be seen from the schematic diagram (Diagram), the reduction in the counts per cc., due to the dilution of the RISA by inactive plasma, is a quantitative measure of the plasma volume (Illustration VII). Actually, if the hematocrit remains constant, as it almost always does

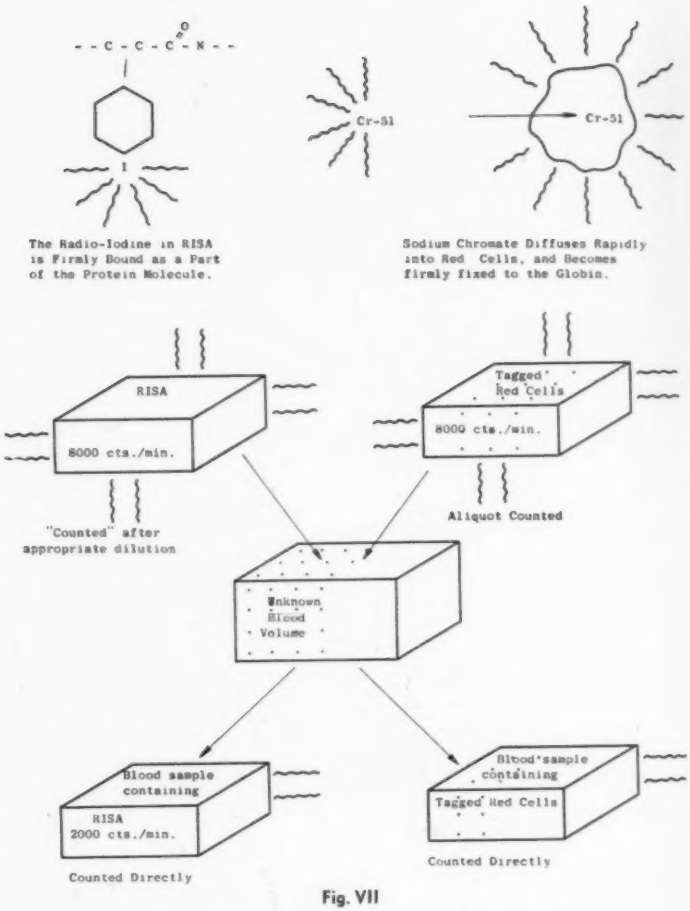


Fig. VII

during the quarter hour required for mixing, one does not need to separate the plasma at the end, but may count the whole blood and thus arrive directly at the total blood volume.

$$\text{B.V.} = \frac{\text{Cts./min./cc. std.} \times \text{vol. std.}}{\text{Cts./min./cc. Blood (measured)}}$$

As has been pointed out, hemolysis does not influence the test which may be carried out before the operation to give a "base line," after the surgery, to give a measure of blood loss, and as

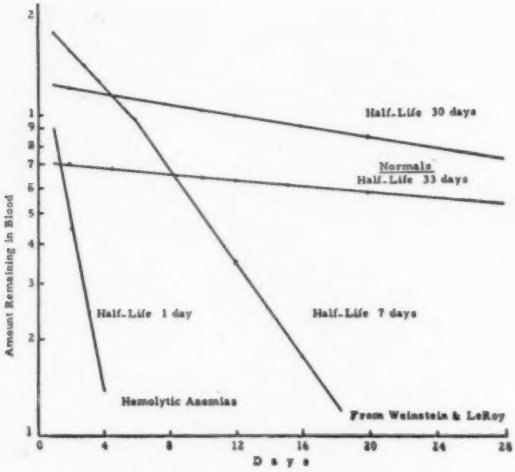
Fig. VIII

many times thereafter as may be necessary to follow the subsequent fluid status of the patient.

The equipment is identical with that used for thyroid uptake studies, and one may wish to employ the very sensitive well scintillator of Illustration IV, to keep the activity used as low as possible.

RED CELL STUDIES WITH CR-51

For the hemotologist, isotopes open up several other fertile fields. On incubating red cells, or even whole blood, with Cr-51 (Rachromate), the activity goes inside the cell, is converted into an insoluble form and so tags these cells for their entire life. A simple technique has been devised for doing this in a single sealed bottle without damage to the cells. One then reinjects (Illustration VIII) a known amount of activity as tagged cells. After mixing, exactly the same principle of dilution may be used to determine the red cell volume or mass. If, then, on subsequent days or weeks, we withdraw identical samples of blood and "count" we can draw a curve from which the life span of the cells may be read (Illustration IX). By this particular technique, the normal half-life is between 28 and 33 days, but in hemolytic anemia will fall far below this value. Studies show that the results very closely parallel the values secured by the elaborate and time consuming Ashby agglutination technique.



RACHROMATE PROVIDES A SIMPLE AND ACCURATE METHOD OF DETERMINING RED CELL MASS, AND FOLLOWING CELL SURVIVAL TIMES IN HEMOLYTIC ANEMIAS.

WITH OUR NEW "ACD VIAL" TAGGING IS ESSENTIALLY COMPLETE IN 15 MINUTES.

Fig. IX

DIAGNOSIS OF PERNICIOUS ANEMIA

I imagine every laboratory has been called upon to determine whether or not one, or more, of its patients, who over the years had had all sorts of therapy, really has P. A., and thus requires active B-12 and intrinsic factor therapy. Thanks to very recent studies in which the author's group has had a part, isotopes now provide a surprisingly simple and accurate answer. An extremely minute dose (0.5 uc.) of Cobalt labeled B-12 is given orally, a 1-2 mg. dose of inactive B-12 is then given parenterally to prevent deposition in the liver, and the 24 hour composite urine, containing most of the B-12 activity absorbed through the gastrointestinal tract, is collected and "counted." If the urine contains less than 4-5% of the administered dose, the indication of P. A. is strong, but may be confirmed by repeating the test, but also giving a standard capsule of I. F. at the same time. Output will then increase to over 10% with an average response of 15-18%, while sprue and similar states will show essentially no response (illustration X).

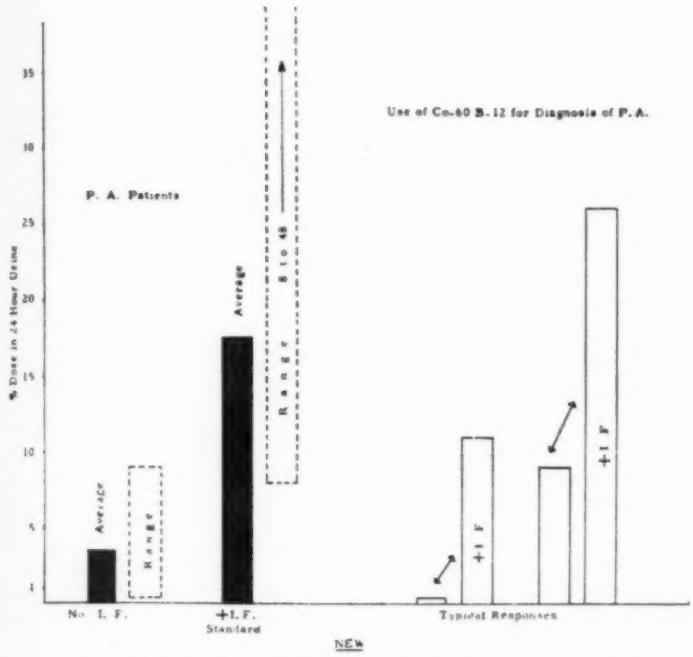


Fig. X

Other Diagnostic Tests

Many other diagnostic tests are in the investigational stage. Among these may be mentioned a very sensitive liver function (polygonal cell) test based upon I-131 tagged Rose Bengal, and a measure of total body water based on 4-iodo-antipyrine, both agents having been evolved by members of our isotope research staff. Both P-32 and RISA have been used to diagnose and localize tumor masses. I-131 together with a suitable "scintiscanner" give a reasonably exact picture of the thyroid shape and position.

Training and Experience Requirements

Realizing the current and future uses of diagnostic radioisotopes, the Atomic Energy Commission has provided that

medical work with radioisotopes is not restricted to research institutions or to radiologists, but often authorizations are given to qualified physicians and to groups working with them who meet certain fundamental qualifications.

The first of these involves training which may be secured either in one of the formal courses given by various qualified teaching institutions, or by private tutoring under a preceptor currently qualified to use the isotope or isotopes in question. For instance a person wishing to do diagnostic radio-iodine work must have the basic technical training enabling him to safely use radioactive materials, and to accurately make the necessary measurements with them. He must have spent 30 hours in such training and must have participated in the clinical management of at least 10 cases. Following this, the treatment of 10 patients makes him eligible to apply for therapy; the carrying out of 3 blood volumes with RISA, 3 red cell taggings with Rachromate then completes his qualifications for these techniques.

Training of Isotope Technologists

Unfortunately, to date, no real facilities exist for the training of the medical technologist. As a result, experience must be gained, gradually, as a part of an active isotope group. As the author has had occasion to see repeatedly in his own laboratory, the careful worker, with the usual background of the medical technologist, or its equivalent in university laboratory science, can master isotopic techniques in not over one or two months of intensive application. The value of such specialized training as an aid to advancement in your field certainly does not need stressing, because for many years to come the demand for isotopic technologists is going to far exceed the supply.

Résumé

This, then, completes our survey of some of the more important facts the medical technologist will need to know about isotopes and their uses. We hope that it has stimulated your interest and that when you return home you will wish to tell your associates about it, and through reading, and, where possible through personal training, become more familiar with the possibilities which it opens up.

PAPER CHROMATOGRAPHY IN MEDICINE*

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Chromatography has been defined succintly by a Britisher as ". . . a technique by which coloured substances can be made to declare their individual presences in a mixture, and by which they can be separated and purified."[1] As applied to present-day chromatography, the specification "coloured substances" must be enlarged to include, as well, colorless substances that: (1) form colored complexes with other chemicals; (2) cause color change in indicators; (3) leave radio-autographs; (4) fluoresce in ultra-violet light.

Although Tswett first realized the analytical potentialities of chromatography as early as 1903,[2] it was not until 1941 that Martin and Synge introduced a revolutionary technic employing two liquid phases,[3] in contrast to previous methods involving primarily adsorption of substances on powdered solids. Their discovery, for which they were awarded the Nobel Prize in Chemistry, freed chromatography from the limitations imposed by a solely adsorptive process and elevated it to first order importance as an analytical tool in chemistry. In 1944, Consden, Gordon and Martin adapted the technic to filter paper;[4] thus, "paper chromatography" was introduced, and with it chromatographic analysis was placed within reach of the clinical laboratory. The great value of chromatography lies in its ability to separate from small volumes of solution, both qualitatively and quantitatively, substances of similar nature that are otherwise inseparable, or are separable only through involved chemical procedures. Medicine has lagged behind other fields of applied chemistry in utilizing chromatography, but recently there has been an increasing number of articles describing its use in the investigation of clinical problems.[5,6,7,8]

METHOD

Producing a Chromatogram: A small volume of solution containing the solutes to be separated is placed near one edge of a piece of filter paper. The edge of the paper adjacent to the spot is then immersed in a vessel of appropriate solvent which, by the operator's choice, either ascends or descends along the paper by capillary action. As the solvent traverses the spot, the solutes contained in the latter go into solution with it, only to be deposited again at distances from the original spot which are more or less characteristic for each solute in that particular solvent. After the

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solvent has advanced a distance that effects satisfactory separation, the paper is dried and treated in a manner necessary for development of color, or otherwise causing the solutes to manifest themselves. The finished chromatogram is then ready for analysis.

In chromatographic parlance a term often referred to is the Rf value of a substance, which is a numerical expression of its mobility across paper in a particular solvent. It is the ratio of the distance traveled by the substance to the distance traveled by the solvent front in the same time, both distances having been measured from a common starting point. Figure 1-A shows diagrammatically how the Rf value is determined. It will be seen, therefore, that a single substance may have different Rf values in different solvents; i.e., the Rf value of methionine in phenol is .73, but in lutidine it is .35 and in butanol-acetic acid it is .55. The Rf value of a substance in a particular solvent is constant and can be reproduced from one determination to the next, provided certain factors are controlled. These have been listed by Consden et al., and include (1) paper, (2) temperature, (3) quantity of solute, (4) extraneous substances, (5) degree of saturation with water and (6) supply of solvent and distance between starting point and source of solvent.[4]

In one-dimensional chromatograms some of the substances being separated may have identical Rf values, or values so similar that separation is unsatisfactory or impossible. When this occurs definition may be obtained by running additional one-dimensional chromatograms with other solvents in which the substances have more divergent Rf values. More commonly, however, two-dimensional chromatograms are run as shown diagrammatically in Figure 1-B. A one-dimensional chromatogram is made and the paper dried until all the first solvent has disappeared. The paper is then turned 90 degrees and a run is completed with a second solvent whose direction of flow is perpendicular to that of the first solvent. Two-dimensional chromatography usually results in definitive separation since it is unlikely that substances would have identical Rf values in each of two solvents.

After the chromatogram has been developed, the substances that have been separated may be identified by comparison with chromatograms of known substances treated in a like manner, or by reference to a table of Rf values, provided conditions of the run were stringently controlled. As experience is gained one finds that spots of familiar substances fall into stereotyped patterns which allow recognition at a glance—as though one were reading a map, so to speak. Templates may be used to facilitate identification of spots, but they are impractical unless conditions of the runs are controlled to prevent changing Rf values from

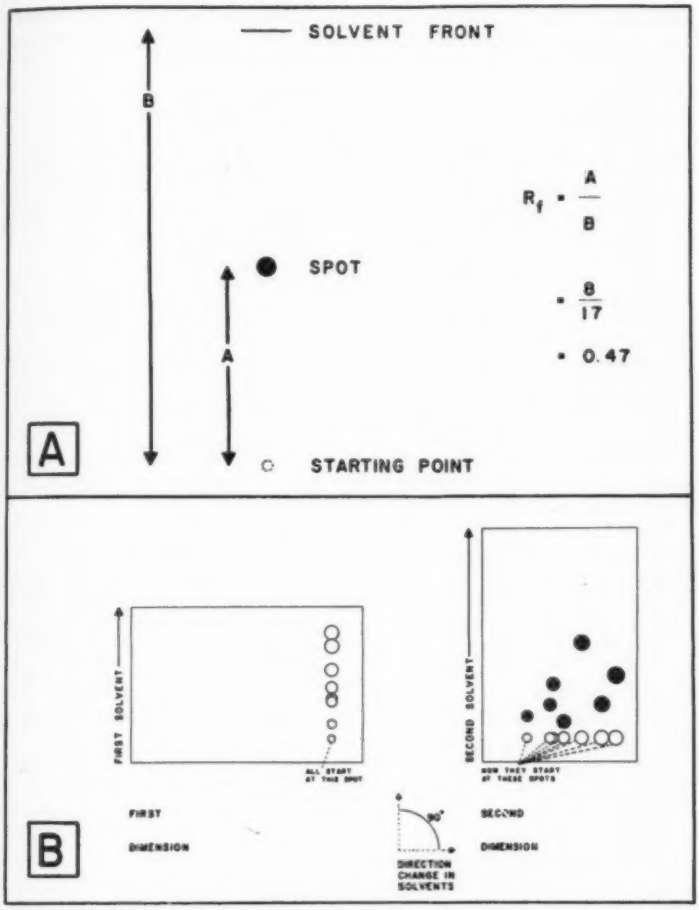

Figure 1

A. Diagram showing how a R_f value is determined.
B. Diagrammatic illustration of a two-dimensional separation of several amino acids.

one chromatogram to the next. The quantity of substance in a spot may be determined in several ways. The spot may be cut out and weighed for comparison with a spot containing a known amount of the substance treated in a like manner. There are

methods available which, in general, involve translation of spot size and color intensity into quantity of substance. These have been described in detail by Lederer and Lederer.[2]

APPLICATION

Our present interest lies in a qualitative study, by means of paper chromatography, of urinary amino acid excretion in patients with various diseases of the liver, particularly cirrhosis and viral hepatitis. The description of technic and materials used is offered as an illustration of how paper chromatography may be applied in the investigation of clinical problems.

MATERIALS AND EQUIPMENT

Paper: Whatman No. 1 filter paper (for chromatography) is excellent. For two-dimensional chromatograms sheets that measure 18½ x 22½ inches are used. These may be cut into strips for one-dimensional runs, or several one-dimensional runs may be accomplished side by side on a single sheet. If one desires to run a large number of two-dimensional chromatograms simultaneously, the large sheets may be cut into squares of smaller size, i.e., 20 x 20 cm., which are much less cumbersome. Several small squares may be affixed in supporting racks made from aluminum or stainless steel, as described by Datta et al.[9]

Cabinet: The Chromatocab manufactured by the Research Equipment Corporation, Oakland, California, is efficiently designed to handle large sheets of paper. It is air-tight, well-insulated and is large enough to accommodate several chromatograms at one time. In addition there is room for vessels containing chemicals to modify the cabinet's atmosphere. If one is using small papers arranged in a rack, a glass aquarium of sufficient size and with a snugly fitting lid may serve as a "cabinet." In such systems the rack is placed in a tray containing the solvent, and the latter ascends the paper.

Phenol Solvent: This is a 4:1 mixture of Mallinckrodt "gilt label" liquefied phenol and distilled water. It is made fresh prior to each run, and is used only once. At room temperature it is completely miscible; when cool it separates into layers, but solution is effected by shaking after gentle warming.

Collidine-Lutidine Solvent: Two volumes of distilled water are added to one volume each of 2,4,6-collidine (Matheson) and 2,6-lutidine (Matheson). The mixture is shaken in a separatory funnel and then allowed to stand until it cools to room temperature. About one-half of the water goes into solution with the collidine and lutidine; the rest forms a layer which is drawn off and discarded. The solvent is used only once; sowever, because of their cost, the collidine and lutidine are recovered by fractional distillation.

Ninhydrin Solution: 0.5 gm. ninhydrin and 20 mg. stannous

chloride are mixed with 460 cc. 98% iso-propyl alcohol and the total volume brought to 500 cc. with distilled water. The solution is fairly stable if kept in a dark bottle and refrigerated when not in use. We have found it advisable to run a test strip to check the reagent's ability to produce color in the presence of amino acids.

TECHNIC

Step 1—Spotting the Paper: On a sheet of paper a point is marked 3¼ inches from each of two sides forming a corner. With a micro-pipette the desired volume of solution containing the amino acids to be separated is run onto the marked point with interval drying so that the final diameter of the spot is about 0.5 cm. This process can be hastened by directing a stream of warm air from a commercial hair dryer against the underside of the paper. The paper is then folded one inch from an edge adjacent to the spot. The resultant crease accommodates the glass retaining rod which holds the paper in the trough of solvent. The paper is next folded 1¼ inches from the first crease and in the opposite direction. The resultant crease accommodates the anti-siphon rod which holds the paper away from the edge of the solvent trough as it descends into the cabinet. The spot containing the amino acids is thus one inch in the descending direction from the anti-siphon rod. If the spot is urine it is now exposed to ammonia fumes for 3 minutes.

Step 2—Equilibration: The sheet is hung in the cabinet by fixing its folded edge in the empty solvent trough so that its shorter dimension is downward. In the bottom of the cabinet is placed a large shallow tray of water and a wide-mouth beaker of phenol solvent. A tall wick of filter paper extends from the solvent into the atmosphere of the cabinet. The lid of the cabinet is closed and the sheet allowed to equilibrate with water vapor and solvent vapor for 3 hours. Adequate hydration of the paper is imperative, but water-logging is to be avoided since it results in diffusion of spots. Large sheets may be hydrated by hanging them in a hood with a steam generator; racks of small papers may be hydrated by placing them in a waste-basket into which steam is fed. We have tried all three methods, and have found that steaming is cumbersome and has no advantage over cabinet hydration.

Step 3—First Dimensional Run with Phenol: Quickly as possible to avoid change in the cabinet's atmosphere, the lid is opened and the trough filled with phenol solvent. In the bottom of the cabinet is placed a wide-mouthed beaker containing a teaspoonful of moistened sodium cyanide to help prevent deterioration of the phenol. If the amino acids are contained in urine, "tighter" spots result if a small volume of 0.3 per cent ammonia water in a beaker is placed in the cabinet. The lid is now closed and is not opened again until the run is completed.

The run may be stopped after a set time has elapsed, or when the phenol front has advanced a desired distance. We stop the run when the front is 1 cm. from the bottom edge of the paper; this requires about 24 hours. The cabinet is opened and a vacuum line run in to evacuate noxious fumes. The sheet is grasped by its bottom edge, inverted and hung by clothespins in a hood. It is dried by a stream of air at room temperature until no trace of phenol can be detected in the paper by smelling it. The edge of the sheet exhibiting the darkened phenol front, as well as the edge which was in the solvent trough, is trimmed off. In the meantime, the cabinet is cleaned and ventilated preparatory to the run with a second solvent.

Step 4—Equilibration: The sheet is turned 90°, and the edge adjacent to and parallel with the amino acids (now separated in one dimension) is folded as previously described. The paper is then hung from the empty solvent trough so that its longer dimension is downward. Equilibration with water vapor and collidine-lutidine solvent vapor is carried out for 3 hours as it was with phenol.

Step 5—Second Dimensional Run with Collidine-Lutidine: The cabinet is opened and collidine-lutidine solvent quickly added to the trough. The lid is closed and not opened again until the solvent front is 1 cm. from the bottom edge of the paper; this requires about 24 hours. During the run with this solvent, cyanide is not necessary in the cabinet; ammonia water is used as in the phenol run if the amino acids are contained in urine. When the run is completed, the sheet is hung in a hood and dried until no trace of the solvent can be detected by smelling the paper.

Step 6—Spraying with Ninhydrin Solution: While the dry sheet is hanging in the hood it is sprayed uniformly with ninhydrin solution from an atomizer attached to a compressed air-line. The paper is then allowed to dry again.

Step 7—Developing the Chromatogram: The sheet is hung in an oven and exposed to a temperature of 90° C. for 7 minutes. A chemical reaction occurs between the amino acids and ninhydrin, and a colored complex is formed. This causes the separated amino acids to become visible as spots. The chromatogram is now finished and ready for analysis. For future reference it may be photographed in color and black-and-white. Methods for preserving the chromatogram itself are described.[2]

RESULTS

Figure 2 presents a series of one-dimensional chromatograms developed with phenol and timed to show the progressive separation of 8 amino acids from a common source. In the experiment, 2 mg. each of glutamic acid, asparagine, serine, threonine, alanine, valine, methionine and phenylalanine were added

to 10 cc. of distilled water. To each of 6 strips of filter paper were added 10 lambda of the solution. Five of the strips were submitted simultaneuosly to a run with phenol solvent using the aforementioned technic. A strip was removed from the cabinet every 3 hours during the first 12 hours of the run; the fifth strip was removed after 24 hours. The remaining strip was not submitted to the solvent run, but was sprayed with ninhydrin to show the concentration of amino acids at the starting point.

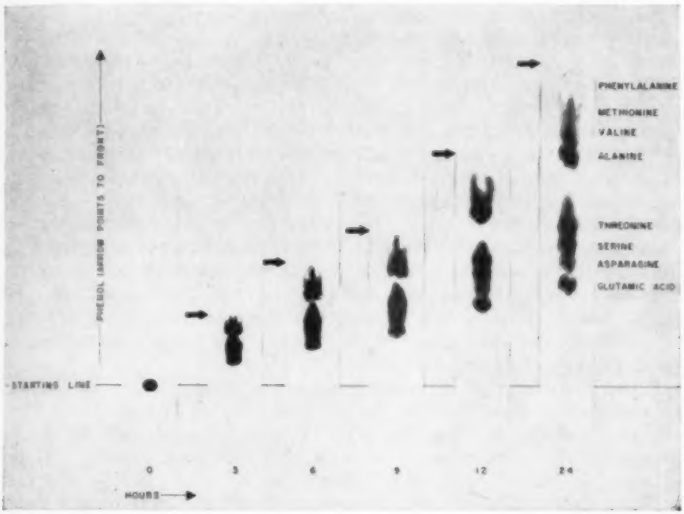

Figure 2

A series of one-dimensional chromatograms timed to demonstrate the progressive partition of eight amino acids in pure solution. The phenylalanine spot was too lightly colored for black-and-white reproduction. There is a "comet" ahead of the threonine spot.

Figure 2 not only shows the remarkable ability of paper chromatography to separate into entities a group of similar substances of small volume of solution, but it also high-lights several points:

1. A solvent run must extend over an adequate period of time, or separation of substances is not effected.

2. In the 24-hour strip discrete spots are visible, but some overlapping is present which would interfere with quantitative analysis. Although the amino acids used were selected because of their significantly different Rf values in phenol, better definition would be attained in a two-dimensional chromatogram.

3. Separation of substances is affected by their concentration. The concentration of amino acids in this experimental solution was purposely higher than would be encountered in physiological fluids. This was done to obtain spots of intense color for photographic reproduction.

4. In successful chromatography, the solute must not be too soluble in the solvent. From the strips it is easy to surmise that excessive solubility of the amino acids in the phenol solvent would have allowed them to advance en masse with the solvent front, thereby precluding their separation.

5. The solute must not be too insoluble in the solvent, for under such circumstances the solute would remain at the starting point while the solvent advances without effecting separation.

6. A solvent which advances too rapidly fails to effect separation because the run is completed before the various solutes are deposited at their characteristic distances from the starting point. To some extent, this inadequacy of rapidly advancing solvents is remedied by modifications which prolong the run; i.e., the solvent may drip off the bottom edge of the paper for awhile, or wicks may be placed in contact with tops of papers in ascending runs. Such maneuvers make it difficult to decide when to stop a run, however, and it is preferable to select solvents that advance more slowly. This is one of the attributes of the widely popular phenol and collidine-lutidine solvents. The requirements of a good solvent have been discussed by Block.[10]

7. If a run continues too long, solutes will eventually be carried off the end of the paper by the solvent. In the 24-hour strip (see Figure 2), it is apparent that phenylalanine would have been lost altogether had the phenol solvent been allowed to drip off the bottom of the strip. Actually, runs do not have to be clocked within exacting limits, but setting a predetermined limit on the solvent front removes another variable from the technic.

Figure 3-A presents a series of one-dimensional chromatograms that compare the urinary amino acid pattern of a normal man with patterns of 5 male patients with various diseases of the liver. In each chromatogram a 50 lambda aliquot of the first morning specimen was used.

This experiment was introduced to show that paper chromatography can delineate the amino acid content of small quantities of urine, thereby proving itself a valuable tool for clinical investigation. Cursory review of the chromatograms reveals several points—all of them enticing subjects for further study:

1. The normal man excreted several amino acids and in considerable quantity. This suggests that one could conduct a project to determine normal variation in urinary amino acid excretion patterns.

2. The chromatogram of the patient with hepatolenticular de-

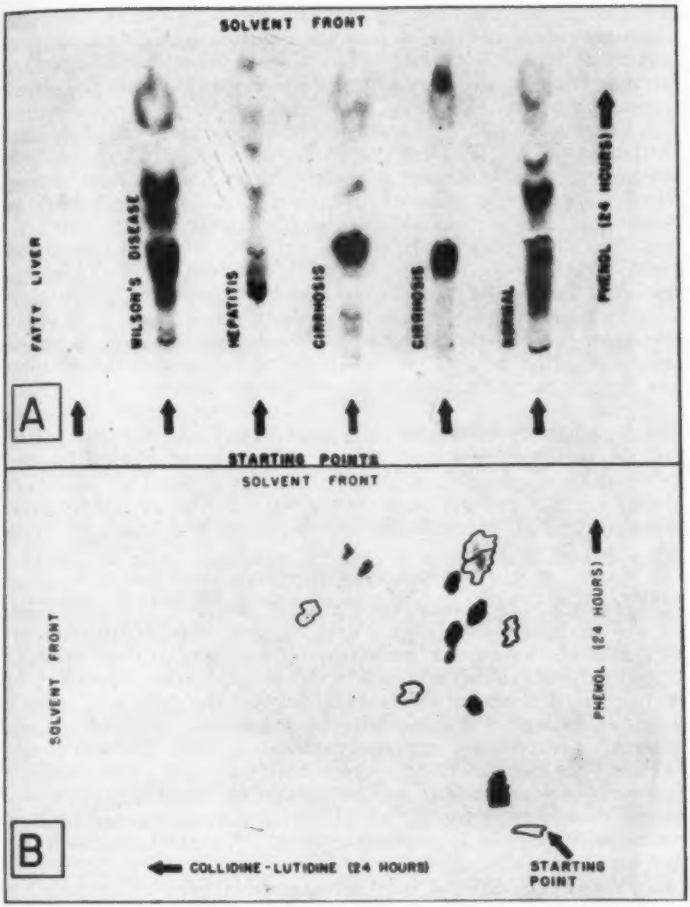

Figure 3

A. One-dimensional chromatograms demonstrating the urinary amino acid pattern of a normal person and of five patients with various diseases of the liver.

B. Two-dimensional chromatogram demonstrating the separation of several amino acids from pure solution. Faintly colored spots have been outlined to facilitate photography.

generaion (Wilson's Disease) demonstrates the abnormally high output of amino acids that is a characteristic finding in the disease. In this respect, the chromatogram has diagnostic significance.

3. The chromatograms of patients with cirrhosis and viral hepatitis reveal that there may be abnormally high excretion of certain amino acids during the course of severe hepatic disease. In this respect, the chromatogram has prognostic, as well as diagnostic, significance.

4. Conversely, the concentration of amino acids in the urine of the patient with fatty liver was so low that scarcely any color was present upon development with ninhydrin. This may be of prognostic significance since the patient, although gravely ill, recovered while the two patients with cirrhosis lapsed into coma and died. The patient with viral hepatitis likewise lapsed into coma, but improved after treatment with ACTH and glutamic acid and eventually recovered. Paper chromatography assumes added versatility in the study of this problem since it affords a means of quantitative determination of specific amino acids excreted. Walshe has written an excellent paper on this subject, and it is illustrated with several chromatograms.[5]

Figure 3-B is a two-dimensional chromatogram of several amino acids in pure solution, and demonstrates the greater efficiency of the two-dimensional technic in producing discrete spots than is possible with the one-dimensional technic. The amino acids were separated from 10 lambda of solution—an amazingly small volume that would preclude isolation by chemical analysis.

COMMENT

In a brief presentation such as this, which may be for some their introduction to chromatography, one tends to oversimplify the subject. Paper chromatography, in the light of startling analytical feats it can accomplish, is simple when compared with more tedious chemical methods. However, the details of technic, in contrast with the simplicity of principles involved, require careful and repeated experimentation for reliable results and finesse. "Cook-book" directions do not guarantee decent chromatograms, because results are influenced by many variables. For example, adequate hydration of the paper—of paramount importance—is less of a problem in humid New Orleans than it is in a more arid climate.

Now largely confined to the research laboratory, does paper chromatography have a place in the clinical laboratory? Surely it does if one is willing to apply it. There are components of biological fluids that we give only passing attention to, because their elaboration is outside the repertoire of the usual hospital laboratory. For example, amino acids are infrequently discussed beyond mere mention in clinical problems, yet they are subject to significant variation in hepatic and renal diseases, two common causes for hospital admissions. Paper chromatography allows the investigation of amino acids at the clinical laboratory level. This

may be true for porphyrins, steroids, sugars, vitamins, anti-biotics, etc. Elaborate equipment is not needed for one's early endeavors in chromatography. To the contrary, adequate one-dimensional chromatograms may be run in test-tubes, and two-dimensional chromatograms may be run in glass jars. Technologists who are belabored by lengthy chemical procedures, or are seeking a method of approach to a problem in analysis, would do well to review books dealing with chromatography.[2,8,10,11] Perhaps they will find a simple chromatographic substitute for more complicated technics. If not an attempt to develop one may be warranted.

SUMMARY

The technic of paper chromatography has been described briefly and significant points illustrated with diagrams and chromatograms. Suggestions have been made for applying the technic to problems in clinical medicine.

ACKNOWLEDGMENT

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THE SIGNIFICANCE OF THE DIRECT AND INDIRECT COOMBS TEST*

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Introduction

The development of the so-called antiglobulin or "Coombs" test by Coombs, Mourant and Race in 1945[1, 2], was one of several factors largely responsible for the tremendous increase in interest in recent years in the field of immunohematology. As a result of this stimulus a great deal has been learned regarding the pathogenesis of anemias which were formerly poorly understood. The importance of this contribution to our knowledge should not be minimized, but nonetheless, it should be pointed out that Moreschi in 1908[3] reported similar observations. He presented experimental evidence that rabbit red blood cells that had been sensitized with a goat anti-rabbit cell immune serum which was too weak to produce agglutination of the rabbit cells, could be agglutinated by washing these cells and exposing them to the serum of a rabbit that had been immunized against goat serum. This pertinent observation was not followed up and even Coombs and his coworkers were unaware of it when they first presented results of their work in 1945. In these initial papers of Coombs and his associates, it was reported that so-called "incomplete" or weak antibodies which were not capable of producing agglutination of the red cells to which they were attached could be detected by adding a washed suspension of such cells to the serum of a rabbit that had been immunized against human serum or human gamma globulin. The antibodies which were firmly attached to the surface of the cells then acted as an antigen by virtue of their being a globulin when they were suspended in a serum containing an antiglobulin antibody. Despite the simplicity of such an explanation, the test is far more complex than this might suggest. The major interest of a group such as this is not so much detailed information about the theoretical aspects of the method nor interpretation of results, but rather how the test can best be performed and what the pitfalls are in performing the test.

Direct and Indirect Coombs Test

There is commonly a misunderstanding on the part of the technologist and occasionally the physician himself as to when one or the other or both of these tests are indicated. Generally speaking, the direct Coombs test is indicated when one suspects the presence of antibody on the surface of the red cells of a patient thought to have an autoimmune hemolytic anemia. The

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indirect Coombs test is requested when one is attempting to detect the presence of antibody in the serum of a patient; it is particularly valuable in detecting antibodies in the serum of a patient who has shown evidence of previous hemolytic transfusion reactions when such antibodies are not detected by ordinary methods of crossmatching.

Method of Performing the Coombs Test

Direct Test. The patient's red cells are washed at least three times and preferably four times in a large volume of normal saline. A 10-15% saline suspension of these cells is then made. The method will vary beyond this point and it is suggested that, if commercial Coombs serum is used, directions accompanying the serum be followed. If an adequately tested serum has been prepared in your own laboratory, then a satisfactory method is to mix one drop of antiglobulin serum with one drop of the saline suspension of red cells on a translucent tile, rock gently from time to time and observe for agglutination with the eye or hand-lens up to a period of five minutes. Normal unsensitized and weakly sensitized red cells should serve as controls. The direct test is not entirely satisfactory for two reasons: 1) it does not give adequate information about the degree of sensitization and 2) not all antibodies react with the same concentration of Coombs serum, that is to say, some sensitized cells may not be agglutinated by an undiluted serum, yet be readily agglutinated by a diluted serum. For this reason it has been suggested that a semi-quantitative method be used when one suspects an autoimmune hemolytic anemia.

Quantitative Direct Test. The Coombs serum is serially diluted with normal saline as follows: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048. A drop of normal saline serves as the control. A drop of a 10-15% saline suspension of red cells is added to a drop of each of the above dilutions of Coombs serum and read after five minutes. The degree of agglutination is graded 0 through 4+.

Indirect Test. If there is a strong suspicion that a patient's serum does contain antibody and the direct test does not indicate the presence of antibody on the surface of the red cells, then it is recommended that the indirect test be carried out. The antibody present in serum is usually of the warm type and as a rule the suspension of normal red cells and the patient's serum are allowed to stand at 37° C for two hours. It is true that rarely the antibody will be of the cold type and if the indirect test at 37° C is negative it can be repeated with acidification of the patient's serum ten percent by volume with N/5 HCl

and placing the suspension of cells and serum at 20° C for two hours before washing. Although acidification does little to enhance the reaction of warm antibodies, it markedly enhances the reaction of pathologic cold antibodies according to Dacie. It is also necessary for complement to be present for the cold antibody to sensitize red cells and therefore the test serum should be fresh.

It is preferable to use about five times as much serum as the red cell suspension (20-30% saline suspension of red cells). After incubation the cells are washed four times with saline and if the cells have been incubated at 20° C the saline should be warmed to 37° C prior to washing to be sure that the non-specific cold agglutinins are eluted.

Cells that have been sensitized by warm antibodies are apt to be most strongly agglutinated by relatively high dilutions of antiglobulin serum (1:16 to 1:64), whereas cold antibodies are best detected by undiluted or low dilutions of antiglobulin serum. For this reason it is best to serially dilute the antiglobulin sera when dealing with an unknown antibody and use the 1:4 and 1:64 dilutions in performing the test so as to have the optimal dilutions for detecting either cold or warm antibodies.

Titration of Antibody by the Indirect Coombs Test. In dealing with warm antibodies the patient's serum may be serially diluted with saline and the various dilutions incubated at 37° C with the cells to be sensitized. In titrating cold antibodies, saline cannot be used but rather normal acidified serum that has been previously absorbed at O° C to remove the incomplete cold antibody that is normally present. The cells and serially diluted serum are allowed to stand at 20° C and at 37° C for two hours, washed and then treated with the antiglobulin serum.

Sources of Error in Technique

A. False negative reactions:

1. Not all batches of antiglobulin sera will be equally potent in their ability to agglutinate red cells sensitized by various antibodies. Crawford and Mollison[4] demonstrated that a particular antiglobulin serum, capable of agglutinating red cells from patients with autoimmune hemolytic anemia as well as cells which had been exposed to incomplete anti-Rh or to normal incomplete cold antibodies, could be absorbed with any one of these types of sensitized cells so that the serum no longer agglutinated the cells with which it had been absorbed but retained its ability to agglutinate the other types of sensitized cells. It is therefore advisable to test each lot of serum to be certain of its potency and then use two such sera for each test.

2. Insufficient washing of red cells. The red cells, if insufficiently washed to remove non-specific, non-antibody globulin that may have adhered to the surface of the cells, may give a false negative reaction. This non-specific globulin is capable of reacting with the antibodies of the antiglobulin serum thus preventing the reaction with the antibody globulin coating the cells. Generally speaking, four washings with saline are sufficient to remove such non-specific globulin, but periodically it is well to check the fourth wash solution with sulfosalicylic acid to be sure no protein is present.

3. Prozone phenomenon or suboptimal dilution. Some antiglobulin sera may exhibit a marked prozone phenomenon in which case red cells coated with antibody may not be agglutinated unless the sera have been optimally diluted. van Loghem and his associates[5] presented evidence to suggest that this prozone phenomenon was due to the excess of a single antibody and that the failure of such an antiglobulin serum to agglutinate red cells was merely due to an excess of antibody such as is encountered in certain precipitation systems and that serial dilution was necessary to make the antibody evident. It has already been pointed out that the cold antibodies are most apt to be agglutinated by antiglobulin sera in low dilutions and in fact, cells sensitized with such antibody may not be agglutinated in a dilution as high as 1:64, a dilution which might well produce maximum agglutination of red cells coated with anti-D antibody.

B. False positive reactions:

1. Inadequately absorbed antiglobulin serum. Unless the antiglobulin serum has been adequately absorbed with normal human red cells, it may produce agglutination of red cells even though they are not coated with antibody. It is usually assumed that all commercial antiglobulin sera have been adequately absorbed although this may not always be the case. If preparing your own antiglobulin serum, it should be absorbed with human red cells that have been washed at least six times in a large volume of saline. The serum should be absorbed first with an equal volume of packed group-O cells and then with group-A,B cells. The absorption should be carried out for at least one hour at 4° C before centrifuging. Such a serum when diluted 1:4 should not agglutinate washed normal red cells but should agglutinate cells weakly sensitized with anti-D.

2. Cold agglutinins present in blood may sensitize cells so that such washed cells may be agglutinated by the antiglobulin serum even though the entire procedure is carried out at 37° C. A much more common source of error is to allow the blood, par-

ticularly clotted or defibrinated, to be placed in the refrigerator prior to testing. As a result the test may well be positive due to the adsorption of a normal incomplete cold antibody present in the serum. Heparinized or oxalated blood is less apt to give such a false positive reaction as the presence of anticoagulants inhibits the adsorption of antibody. The Donath-Landsteiner antibody will also sensitize red cells for the antiglobulin test. One means of differentiating between such a false positive cold antibody and a warm antibody is to add a minute amount of human gamma globulin to the antiglobulin serum. Such a serum loses completely its ability to agglutinate cells coated with a warm antibody yet it retains its ability to agglutinate the cells coated with the cold antibody. The logical conclusion is that the warm antibody is a gamma globulin, whereas the cold antibody is not.

Conclusion

The antiglobulin test offers a challenge to the technologist in that under certain circumstances red cells coated with antibody may give negative reactions and that cells not coated may give positive reactions. The person performing the test must have an understanding of the basic problem as well as being aware of the sources of error in carrying out the test if optimal results are to be obtained.

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A MICRO-METHOD FOR THE SERODIAGNOSIS OF RHEUMATIC FEVER (ANTI-STREPTOLYSIN-O-TEST)*†

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The serodiagnosis of rheumatic fever dates back to 1932 (Todd and coworkers, [9, 10, 11]). The isolation and purification of the "oxygen-labile Streptolysin O" by Todd (12) and Smythe and Harris (7), and finally its production in dried (lyophilised) form by Rantz and Randall (3, 4) made possible the laboratory rheumatic fever diagnosis. The availability of "Streptolysin O Reagent," commercially produced by Difco Laboratories, and the simplification in technical procedure of test-methods by various authors have made possible the performance of tests even in smaller laboratories. The still high price of the reagent led us to try out a method which would allow economy of reagent without complicating routine test procedures or endangering reliability of test. The method worked out is given below.

Principle of Method

Instead of using quantities measured in ml by use of pipets, as done in routine procedures, we make use of standardized droppers to deliver drops of constant size (Rappaport and Stark, 5), and reagent, sera, buffer solution and red cell suspension are delivered by dropping instead of pipetting.

Manufacture of Droppers

Two kinds of droppers are used, one delivering 1 ml in 16 drops (designated 1/16), the other in 20 drops (1/20). One drop of the 1/16 dropper contains 0.0625 ml, of the 1/20 dropper 0.05 ml.

These droppers may be prepared in the laboratory. Glass tubing of about 5 mm outer diameter are drawn to a tip at one end in the flame of a bunsen burner. The tip is cut so that the opening measures 1 mm or less in diameter. The tip is slightly bent.

The droppers are standardised as follows: water is dropped from the dropper into a cylinder made out of a graduated pipette closed at one end. The number of drops required to fill a certain volume is counted. If it is larger than required, the tip of the dropper is narrowed in the flame; if the number of drops is too small then the tip is ground off. Finally the number of drops required to fill 10 ml is counted, and it should be within a limit of $\pm$ 1 drop of the calculated amount i.e. 200 with the 1/20 dropper, 160 with the 1/16 dropper (5).

* This paper is dedicated to Prof. J. Kleeberg in honour of his 60th birthday.
† Reprinted with permission from Acta Medica Orientalia, Vol. XIV, No. 2-3, February-March, 1955.

Reagents

I) Bacto Streptolysin O Reagent (Difco) supplied in ampuls as a dried powder, to be dissolved in 10 ml solvent (see below; reagent II). It allows testing of 10 sera and setting up necessary controls in our test method.

II) Solvent.

$Na_2 SO_3$ (sodium sulfite) ..... 1 part
$NaHSO_3$ (sodium bisulfite) ..... 2 parts

are mixed and stored on a dark place in a well stoppered bottle.

Just before use the content of one bottle Bacto Streptolysin O Reagent is dissolved in 10 ml icy-cold distilled water, in which a knife point quantity (approx. 0.015 Gm) of the a/m sulfite mixture had been dissolved. The unused dissolved reagent is put immediately into the deepfreezer and may be kept firmly frozen up to a fortnight without loss of activity. (In case the whole content of the bottle is used at once + 10 tests—the addition of the sulfite mixture may be omitted, and the reagent diluted in 10 ml of distilled water, as directed on the label of the bottle.)

III) Buffer solution.

NaCl ..... 4,20 Gm
KH_2PO_4 anhydr. ..... 3,17 Gm
Na_2HPO_4 anhydr. ..... 1,42 Gm
NaN_3 (sodium anide) ..... 0.10 Gm

The buffer solution is made up by dissolving 8,8 Gm of the above mixture (Cuevas, 2) in one ltr. of distilled water. The pH has to be adapted to 6,5—6,7 with NaOH solution. The Buffersolution keeps well when stored at 4°C in the refrigerator.

VI) Red Cell suspension. Human red cells of group O are used. Blood is taken from the vein or the fingertip into citrate solution. The red cells are washed three times in the Buffersolution, and finally made up in this solution to a 5% suspension.

Serum Dilutions:

The sera to be tested are diluted in buffer solution (reagent III). One drop of serum (0,05 ml) is needed. The serum should be separated from the clot as soon as possible, and stored at deep temperature in the frozen state. Three stock dilutions (A, B, C) are needed for each serum, which are prepared in the following way:

A pipet of 3 ml capacity is marked with a grease pencil at 0,45 ml, 1,50 ml and 2,55 ml. For each serum 3 test tubes are set up in a rack, the first row tubes show the number of the respective serum and the designation A, the tubes in the second row are marked with the letter B, in the third row with the letter C. Each set of 3 tubes is now filled from the marked pipet with buffer

solution (reagent III) in the following way: tube A gets buffer-solution up to mark at 0,45 ml, tube B gets up to mark 1.50 ml from the mark 0,45 i.e. a quantity of 1,05 ml, and tube C up to mark 2,55 from the mark 1,50 i.e. 1,05 ml. Now with the 1/20 dropper one drop of serum is added to tube A. After mixing 3 drops are transferred to tube B from tube A. From tube B after mixing 4 drops are transferred to tube C, and the first 5 tubes of the test proper are filled as seen in scheme 1). Then from the tube C the second 5 tubes of the test proper are filled as described.

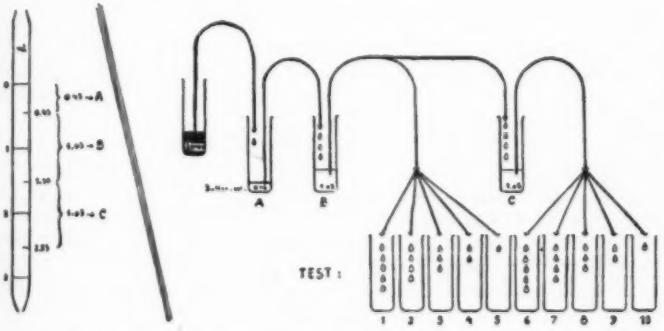
The tubes A contain serum dilutions of 1:10, tubes B 1:80 and tubes C 1:500.

(In case that even 0.05 ml are not available from a serum, the test may be set up in this way: tube A is omitted altogether. 0,02 ml serum is drawn up into a Sahli pipet up to the mark and delivered into a test tube containing 1,6 ml buffer solution. This gives a dilution of 1:80 and, thus, corresponds to tube B. Tube C is then set up as described).

Performance Of Test

The test proper is set up in test tubes 75x8—9 mm (Wasserman test tubes) in the manner shown in scheme 2). Serum dilutions and buffer solution are dispensed by the 1/20 dropper, Streptolysin O Reagent and red cell suspension from the 1/16 dropper.

Scheme 1). Serum-Dilutions.



After the tubes have been filled with the serum dilutions, the buffer solution and the Streptolysin O reagent, the rack is shaken and incubated in a waterbath (or incubator) at 37°C for 15 minutes. Then the red cell suspension is added and the rack incubated at 37°C for further 45 minutes. After 15 minutes from

begin of this incubation period the rack should be removed from the incubation and shaken for 1 minute and then the incubation should be continued. After incubation the rack should be set aside for 30 minutes at room temperature before reading, to allow unhemolysed cells to settle. The antistreptolysin O titer is the highest dilution of serum causing no hemolysis of the red cells.

Two control tubes should be set up, one Streptolysin control, and one Red cell control, as shown in scheme 2; the first control should show complete hemolysis, while the second control should be unhemolysed.

Scheme 2)
Test Proper

TEST TUBE NO.:	1	2	3	4	5	6	7	8	9	10	11	12
From B 1/20 drops	5	4	3	2	1	...	...	...	...	...	...	...
From C 1/20 drops	...	...	...	...	...	5	4	3	2	1	...	...
Buffer solution 1/20 drops	0	1	2	3	4	0	1	2	3	4	5	7
Streptolysin O Reagent 1/16 drops	2	2	2	2	2	2	2	2	2	2	2	...
	Incubate at 37° C. for 15 minutes											
5% Human Red Cells (Group 0) 1/16 drops	2	2	2	2	2	2	2	2	2	2	2	2
	Incubate at 37° C. for 45 min. (Shake after 15 min.) Let stand at room temperature for 30 m. Read.											
Antistreptolysin (Todd) Units	80	100	133	200	400	500	625	833	1250	2500	Lysin/Red Cell Controls	

Reading of the Test:

Hemolysis in the test tubes means absence of substances in the serum which could interfere with the hemolytic activity of the Streptolysin O reagent. No hemolysis means inhibition of the hemolytic action of the Streptolysin O reagent and points to the presence of substances in the serum tested, which interfere with this reaction. These substances are antibodies, made by the body in response to antigenic activity of hemolytic streptococci and represents a specific immunologic antibody reaction of the body.

Interpretation of Test:

Swift (8) states presence of antibody (Antistreptolysin O) in at least 90% of cases who had suffered from streptococcal infection, but as far as the level of the titers is concerned, no agreement is seen amongst the different authors as to its significance. The test is, therefore, of value only in connection with clinical findings. Still it may be stated with Breese (1) that titers over 50 Todd units (9) are definitely pathognomonic. In our experience 100 units seem significant.

As the antibodies, however, show tendency to remain present for weeks and months, the test does not give any hint as to the activity of the illness. The test may mainly be looked at as an exclusion test for rheumatic disease, if negative. On the other hand follow up of cases of suspected rheumatic fever by the

test, may definitely exclude or confirm the clinical diagnosis, when the test is performed at weekly intervals for some weeks. Fall or rise of antibody titer can in these cases give diagnostic as well as prognostic aid. Cortisone, ACTH and treatment with antibotica may somehow influence the test, as several types of antibodies (monovalent, bivalent) appear in the course of the rheumatic disease, similar to our observations (Rappaport and Stark, 6) in Salmonellosis, Rickettsiosis and Brucellosis. (Details on this phenomenon will be given in a later communication.)

Summary

A serodiagnostic procedure is outlined which allows determination of Antistreptolysin O titer from a single drop of serum, by a micromethod, using smallest amounts of stabilized reagents.

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Addendum: From experience we have learned to economize the method further, thus allowing to perform 20 tests from one bottle, as compared with 10 tests in the above method. The new modification is done this way: the tubes A, B, and C, are prepared as described. Now the test proper is set up in two consecutive parts. First: for each serum one test tube only is set up by dropping 5 drops from tube C, corresponding to tube No. 6, and the test is performed and read. Then: when a) *hemolysis* is observed in this tube, the tubes Nos 1 to 5 are set up (from tube B), this means that only the *left* side of the original test procedure is performed. When b) *no hemolysis* is seen in this tube, the tubes Nos 7 and 10 are set up (from tube C), this means that only the *right* side of the original method is performed. Thus only half of the tubes are necessary, and the reagent needed is halved.

THE ROLE OF THE LABORATORY SUPERVISOR

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When thinking about this article, my first impulse was to indicate, in view of years of personal experience, just what hospital laboratory supervisors do, or perhaps, of more importance, what they wish to do. Not desiring to depend entirely upon my personal opinion, I consulted either in person, or by writing, three pathologists, one college instructor of medical technology, two Sisters and three lay laboratory supervisors, as well as several co-workers. In addition, I wrote to the Registry of Medical Technologists in Muncie, Indiana, and to the American Society of Medical Technologists in Houston, Texas, for any material that might prove helpful. I scanned a number of journals for articles relative to the laboratory and perused a few books dealing with education, management, responsibility, job description, departmental organization in hospitals, etc. Not too much has been written pertaining to an administrative laboratory supervisor's job, as such. Personal views were augmented, however, by several of the articles read, as well as by the replies I received from everyone I contacted, all of which tended to broaden my view of the subject; it also made me wonder if anyone exists who could fill the position completely.

One universal opinion gathered from the above sources was to "keep everyone happy." Acting on a second impulse arising from this general agreement, I intend to build this entire article around the possibilities of an administrative supervisor doing just that, keeping everybody happy in his relationship with the laboratory department. This can be done most effectively by constant efforts in maintaining and in giving efficient and accurate service.

Who is this administrative supervisor? To whom is she responsible for the fulfillment of her duties? What qualifications are necessary for one in her position? First and foremost, she is not the director of the laboratory. A qualified pathologist holds that office. This doctor may be on a full or on a part time basis depending on the availability of such specialists in the vicinity, as well as upon the size of the hospital. In this pathologist rests full authority and responsibility for the organization and the operation of the department. In the performance of his duties, the busy pathologist finds a definite need for a chief medical technologist, who may assume the supervision of all other laboratory personnel, and to whom may be delegated certain administrative duties. The administrative angle implies initiative and leadership as well as supervision. Such an administrative individual must possess one quality above all others—the willingness

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to accept responsibility. Confidence based on knowledge of one's natural or acquired ability is an invaluable asset, and is acquired by anyone who has built competency in her profession on a sound educational foundation, and who is motivated to use her knowledge and her skill primarily for the benefit of those whom she serves. For all hospital personnel, the one ultimately served is the patient.

It follows that the said supervisor is the chief technologist, and that she is directly responsible to the pathologist for the fulfullment of her duties. She has acquired, through the practice of her profession, the ability to perform those chemical and biological tests which have been instituted to diagnose and to control disease. It is a misconception, however, that laboratory work is just technique, and that it can be reliably carried out by technologists without adequate supervision. There is no laboratory which can properly function unless it is organized for efficient service. Such organizing connotes supervision of the entire unit by a person who is capable, professionally and technically, by virtue of personality, education, and experience, of managing the department's activities, of training and supervising all its personnel, and of co-operating with every other hospital department.

Relationship with Laboratory Personnel

The administrative laboratory supervisor's first responsibility is to the chief of pathology, whom she approaches freely as a consultant in unusual problems arising in any of the departments of the laboratory. Keeping him informed makes for a better relationship with all concerned. It is not necessary that he be bothered with too many petty troubles and disturbances. Primary decisions as to the specific means of carrying out orders and the specific techniques to be used are his responsibility. This does not relieve the supervisor, however, of considering any procedures which come along from time to time, and of evaluating them, to determine whether they will apply to her own laboratory. The pathologist is happy to discuss these techniques with his chief technologist from the viewpoint that there are new procedures which can possibly save the technician's time, which give better results, and which fit into the laboratory program very nicely. A good example of such an instance is in the performance of a Rh determination. Five years ago, it took one hour to do such a test; the newer procedure enables the technician to do it in one minute.

New products are likewise considered. Often they can save money and time, and give more accurate results; the advice and counsel of the pathologist is of immense value to the supervisor in determining which of these products will fit into the program of the laboratory. Once they have been decided upon, the sales-

men should be allowed to detail the product to the personnel who will use it. He knows his product, he knows how to use it, and he should be permitted to explain and to demonstrate the said equipment.

Medical Technologists

Everything should be done to establish a good working team with the departments' technicians. The supervisor considers the requirements for the various sections of the laboratory, such as hematology, chemistry, bacteriology, histology, blood bank, etc. Technologists, properly selected by her to fulfill these requirements, and in sufficient numbers, are indispensable toward the achievement of a smoothly functioning and continuously improving department. Specialization of the technicians in various fields of their work is productive of a greater selection of laboratory tests and of greater accuracy. The responsibility for the development and the functioning of a section in the laboratory will bring out the best in an ambitious technician. The supervisor should be fairly conversant and familiar with all of the techniques used if she is to give over-all supervision. Once the technologist is employed, and assigned, the supervisor shows that she is relied upon, is trusted, and that her interests are always being considered in the way of health, hours, salaries, etc. The technologist is further made to feel that she is a vital part of the functioning of the laboratory, and that as a member of the hospital's personnel she is entitled to certain privileges.

The administrative supervisor will find it most helpful to be democratic in her views with her personnel rather than to be autocratic. Meetings within the department are indispensable in discussing the work of the laboratory; the views of all the medical technologists must be considered, and put to use whenever possible in improving the quality and quantity of service. It is only reasonable to assume that several brains are better than one when deciding certain aspects of the laboratory's functionings. Management will not slip from the supervisor's fingers by virtue of recognizing ability in others, nor by giving due recognition to it. If the supervisor shows that she respects those working under her, they in turn will respect her as a fair and just individual. The supervisor sets the example in being active in her professional societies. The ethics of the profession of medical technology are posted in a conspicuous place, and it is expected that all technologists live up to this code. Working and cooperating in this way with her fellow workers will convince the technologists that they have in their supervisor a helpful advisor, both in personal and in professional matters.

Clerical and Non-Professional Personnel

The stenographers and the clerks do much toward good service by recording results accurately and reporting them promptly.

Proper training and supervision makes of them a real asset in dealing with the medical staff. Giving reports on time and answering a telephone courteously makes for a good feeling. Here it may be added that the chief of staff and the laboratory supervisor should together settle such matters as announcements of new techniques, routines to be performed on all admittances, and the method of settling various questions which may arise with regard to medical staff-laboratory relationships. Dependable results must be given to the staff; the establishment of a laboratory's reputation for accuracy and speed is largely a matter of good supervision. The supervisor shows by her manner and by her actions that she considers constant checking of results of vital importance, and that much of her time, labor and thought are consumed in the frequent checking of reagents, standards, stains, as well as techniques. Such dispositions build up the confidence of the staff in the hospital laboratory.

Non-professional personnel play a responsible part in the accomplishment of laboratory work. Job descriptions for this type of employee should be written and posted by the supervisor. Definite responsibilities are entrusted to them. Every supervisor knows the delight of having a worker who puts every tube away in its proper place, who makes cotton plugs of just the right consistency, who prepares ample sterile supplies, who sharpens needles as carefully as if she herself is to be victimized, and who does the other countless routines entrusted to such employees. It is extremely important that these assistants be properly instructed in their duties; when their job is well done, proper commendation should be given. Words of encouragement and of praise on the part of the supervisor will frequently be the only means necessary to keep such employees contented, happy and stable for many years in the service of the laboratory.

Housekeeping, including orderliness and cleanliness, together with dish-washing, can sometimes create a real problem within the department. It seems there is more of a turnover in this phase of the laboratory personnel than in any other. If good supervision exists, the maid and dishwasher are treated as important people—certainly, no other employee is more missed when absent from duty. A thorough job analysis is necessary, with explanations as to why each duty must be performed perfectly. If the employee expects to wash bloody tubes, to discard smelly pathology specimens, to clean the autopsy room after use, etc. she does not feel that she is being imposed upon when such duties come her way. The technologist can do much to keep the non-professional workers happy by keeping working material in order, and straightening up after themselves. And there are times when the technologist has more time at her disposal than the maid. Good relationship, at such times, prompts a generous hand

in drying slides, in emptying the autoclave, putting away supplies, etc.

Relationship with Hospital Administration

The administration of the laboratory department is entrusted to the pathologist, and through him, many of the administrative duties are transferred to the laboratory supervisor. She acts in turn, as liaison officer between the pathologist and the hospital administrator. The supervisor should be thoroughly familiar with the hospital code of ethics. Any knowledge obtained through laboratory reports that might concern any principle of this code should be reported immediately to the hospital administrator. The supervisor likewise keeps the administrator familiarized with the nature of the laboratory work being done, with the need of competent personnel, and with the value of good equipment. It is the supervisor who advises the need of specialized equipment. Consolation may be had in considering that the more expensive pieces have a long and useful life, and that frequently a single instrument will be the key to many diagnostic aids.

A definite program of buying supplies should be worked out with the stock room management. It is unreasonable to expect the average purchasing agent to have sufficient information or knowledge to purchase many of the laboratory items. Much of this equipment is highly technical, and it is important that the administrative supervisor be certain that she gets the proper chemicals, stains, reagents, glassware, etc. Substitutes bought by a misinformed purchasing agent may be poor economy. Making delicate determinations that may mean life or death justify securing the best equipment available. The final purchasing, of course, can be done through the stock room management, but the salesman should be allowed to present themselves in the laboratory to discuss any new procedures, their new products, or any new ideas they may have for the laboratory.

The proper charging of laboratory work and the mechanisms involved are worked out between the supervisor and the fiscal officer. The methods of charting as well as any changing of laboratory forms should be discussed with the record librarian. The personnel office is kept informed of all time schedules, reasons for absences, vacation, days off, etc. Mutual discussion of the problems that may occur with any of these departments leads to a splendid interdepartmental relationship.

Cooperating and working with the payroll supervisor helps in the adjustments of salaries. A real problem faced by hospital administrators is that of holding competent personnel once they have been obtained. The setting of several distinct salary levels for technologists is advantageous. Certainly, the graduate from the non-approved schoool, who may have to be hired as a result

of the shortage of technologists, should not start on the same salary level as one from the recognized school. Advancement from one level to another should depend on type of experience and achievement, and not on tenure. The almost microscopic increases now accorded at designated time intervals exercise little staying influence on the professional employee. Increases based on tenure are desirable, but salary differentiation sharp enough to indicate recognition of accomplishment is even more important.

Relations with Nursing Service

The ability of the laboratory personnel to have good relations with the nursing staff, thereby harmonizing patient service, is due in great part to the supervisor. This may be brought about by clearly written directives placed on all nursing units with regard to the preparation and the collection of specimens, and also by being exact in informing the floor of any special changes, so that errors and resulting hard feelings may be avoided.

With the student nurse, the supervisor takes a special interest in explaining the reasons and the necessity for properly obtained specimens. She is generous in offering her services to the school-office, as instructor in microbiology, or such. Teaching such a class gives the laboratory supervisor a wonderful opportunity to put over fundamental laboratory principles, which ultimately lends to student nurse cooperation in dealing with the laboratory.

Training School for Medical Technologists

The institution of a training school for medical technologists should be strongly encouraged by the administrative laboratory supervisor in any hospital which has adequate facilities and instructors. The shortage of qualified medical technologists is of grave importance, and loyalty to her profession will prompt the experienced technologist to help in the training of new recruits. Such a school should by all means fulfill the requirements of the Council of Medical Education and Hospitals of The American Medical Association and the Registry of Medical Technologists (A.S.C.P.).

The supervisor examines the credentials, interviews, and accepts students for the school. The ability to stimulate such students with a love for their profession, as well as the ability to train them in Christian courtesies to the patient are prime requisites in the laboratory supervisor. She plans and supervises the course of instruction, assigns their duties, and evaluates and grades the performance of the students. She further provides and encourages an environment in which all interested recruits who meet the qualifications in education, personality, and health, are inspired to uphold the tenets embracing the science of medical technology. This is done without discrimination because of economic level, or of ethnic or religious background. In fine, the

supervisor builds up an appreciation of the profession of medical technology and inspires the prospective student to real scientific accomplishment.

Conclusion and Summary

There seems to be no end to what laboratory supervisors can aspire. It is seen from the above, however, that it is very important for a happy, peaceful, and smooth-running department that the supervisor has not only the ability to do what is required of her, but does it in a way that will "keep everybody happy," and that will demand the respect and esteem of the entire hospital staff. "In happiness, there is peace, and in peace, there is strength."

In summary: 1. The administrative laboratory supervisor should be in charge of the entire laboratory, and have the final word on equipment and supplies, with the advice and the counsel of the pathologist in charge of the entire program. 2. The pathologist's contact should be directly with the laboratory supervisor. Ideally, this supervisor should, as far as possible, act as liaison officer between the pathologist and the hospital administration. This leaves the pathologist time to act as a liaison between the hospital and the medical staff in problems concerning laboratory medicine. 3. Intra-departmental meetings are necessary aids in problem discovery, problem solving, increased acceptance of decisions, and improved ability to execute decisions. 4. The supervisor should familiarize herself with all current laboratory journals, and with other medical articles pertaining to laboratory procedures in the diagnosis and in the control of disease. In this way, she keeps abreast of the advances in her profession. 5. The administrative laboratory supervisor encourages an efficient, speedy and economic department by a service that is courteous, pleasant and complete.

PULMONARY MONILIASIS
THE TECHNIC OF MYCOLOGICAL DIAGNOSIS AND THE PRESENTATION OF FIVE CASES*

M. SUE IVENS, B.S., M.T. (ASCP) and J. B. HUTCHESON, M.D.†

The medical literature during the past several years has contained several articles dealing with broncho-pulmonary moniliasis. This condition has been described as a primary disease entity, secondary to chronic pulmonary disease such as cancer or tuberculosis, and as a complication in the use of broad-spectrum antibiotics such as terramycin and aureomycin.

We have not had sufficient experience at the Lewis-Gale Hospital to enable us to substantiate or refute the work of those authors who believe that moniliasis is not an uncommon disease of the lung. It is the purpose of this paper to discuss briefly five cases of pulmonary disease in which the diagnosis of moniliasis seemed to be justified. In three of the cases repeated cultures revealed pathogenic strains of Candida albicans in the sputum or bronchial aspirate, other possible causative agents were excluded as far as possible, and in two cases C. albicans were demonstrated in the lungs at autopsy. The manner in which the fungi were identified will also be discussed.

Report of Cases

Case 1. The first patient was a 38-year old pregnant, white, female who was hospitalized because of a diffuse, patchy consolidation of her lungs that was demonstrated on a routine x-ray examination. She had a moderate cough but no additional symptoms. Two sputa were negative for malignant cells, five were negative for tuberculosis, and two were positive for a pathogenic strain of C. albicans. Following a negative skin test to an autogenous vaccine of the fungus, potassium iodide was begun in November, 1953. The patient has continued to take this drug to the present time. A marked improvement of the x-ray pictures and a disappearance of the cough were noted one month following the initiation of treatment.

Case 2. The second case was that of a 63-year old colored male who was admitted after becoming unconscious while at work. Chest x-rays revealed soft tissue densities in both lungs which remained more or less constant during the two months of observation. Six smears and cultures of sputa were negative for acid-fast bacilli; however, pathogenic C. albicans were found in smears and on cultures of six sputa and one bronchial aspirate.

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† From the Departments of Clinical Pathology and Bacteriology of the Lewis-Gale Hospital in Roanoke, Va. (Dr. Hutcheson has since removed to Baylor University Hospital in Dallas, Texas.)

Eight smears for malignant cells were negative. A punch biopsy of the lung revealed no specific changes. An exploratory operation of the brain failed to show evidence of a tumor. After a negative skin test to an autogenous vaccine was obtained, potassium iodided was given. The patient failed to improve, and a short time after leaving the hospital a firm nodule appeared over his left clavicle which could have represented a spread from a malignancy of the lung. He died at home and an autopsy was not obtained.

Case 3. A 55-year old white female had been hospitalized in January, 1953 for what was diagnosed as "flu" with definite evidence of pneumonia by x-ray. Three months later she began complaining of cough, fever and weakness. An x-ray revealed marked bronchial thickening for which she was successively given penicillin, aureomycin, streptomycin, and sulfonamides without improvement. She developed a fever of 106° associated with headache, nausea and vomiting. Yeast-like organisms were repeatedly demonstrated in the sputa and were identified as pathogenic C. albicans. Routine febrile agglutinations were negative, as were repeated examinations for tubercle bacilli. A complement-fixation study for moniliasis was negative, but a skin test with an autogenous vaccine was strongly positive after 24 hours. A tentative diagnosis of pulmonary moniliasis was made and desensitization was begun. She developed febrile reactions to the vaccine about two weeks later and became unable to tolerate the weakest strength of the diluted vaccine. She exhibited an idiosyncrasy to potassium iodide. She was studied at the University of Virginia and later at the Jefferson Hospital in Roanoke. In both hospitals, monilia were isolated from the sputum; however, no fungicidal treatment was administered as the clinicians persisted in the idea that the monilia were secondary invaders. The patient now has extensive scarring of her lungs and is following a progressively downhill course.

Case 4. The fourth case involved a 40-year old white male who had had lymphosarcoma for four years. At the time of his last admission he complained of severe weakness and marked anemia. X-rays revealed extensive consolidation of the left lower lung. He was given aureomycin and penicillin for ten days and received ten transfusions of whole blood. At autopsy the area of consolidation in the lung was literally teeming with pseudohyphal elements resembling C. albicans (Fig. 1). The air spaces of the lung showed consolidation by inflammation due to x-ray treatment (Fig. 2). The remaining organs showed widespread involvement by lmyphosarcoma.

Case 5. The last case was that of a one-month old male infant who had erythroblastosis at birth. He received repeated blood transfusions and began to gain weight in a normal fashion. About

three weeks after birth he was admitted to the hospital because of fever, irritability, and failure to eat. His tongue and throat were markedly red and he had cyanosis and labored respirations. He failed to respond to oxygen, cortisone, penicillin and terramycin and died two days after admission. At autopsy (Fig. 3) the kidneys were swollen and contained a large number of light-colored slightly raised abscesses throughout. (Fig. 4) This is a photograph of one of the abscesses showing the abscess wall and interior. These abscesses were infiltrated by large number of chronic inflammatory cells and a few neutrophiles. (Fig. 5) Some yeast-like organisms were thought to be present but were not conclusively identified. The lungs (Fig. 6) showed multiple areas of hemorrhage. Microscopically, lung abscesses were demonstrated which contained branching pseudo-hyphal elements resembly C. albicans (Fig. 7). Higher magnification (Fig. 8) shows the fungi in greater detail.

Mycological Identification

Because of their small size, similarity to gram-positive cocci, and lack of outstanding microscopic characteristics, fungi will not be found in the sputum unless technologists are conscious of the possibility that they might be present (Fig. 9). On direct unstained wet-preparation of fresh sputum, feces, or other material, monilia appear as varying-sized, oval organisms that have thin walls and are somewhat smaller than red blood cells. Budding forms and pseudo-hyphae may also be found. The organisms are gram-positive and may be mistaken for bacilli. If organisms showing these characteristics are demonstrated, it is necessary to perform differential studies to show whether or not C. albicans is present. The studies are indicated by this chart (Fig. 10) which shows the reactions of Candida to Sabouraud's glucose agar, blood agar, Sabouraud's glucose broth, Littman oxgall agar, and the carbohydrate extract broths. On Sabouraud's glucose agar (Fig. 11) or Littman's oxgall agar at room temperature, growth becomes apparent in two to four days as creamy, moist, shiny, flat colonies which measure from 5 to 10 mm. in diameter. A yeast-like odor can be detected when the Petri dish is opened. This represents a 4-day growth on Littman oxgall agar.

A suspension of cells is prepared in sterile saline from a representative colony which can be used as an inoculum for subsequent examinations. Next the surface of a cornmeal agar slant or plate is cut by the inoculating needle. After six to ten days at room temperature on cornmeal agar, pseudo-mycelia are produced which are seen as elongated cells with numerous lateral projections, the blastospores, at points of construction. (Fig. 12) C. albicans produces large, thick-walled chlamydospores on this agar while other species of Candida do not, (Fig. 13). These can be observed under the low-power objective with the culture dish

Figure 1

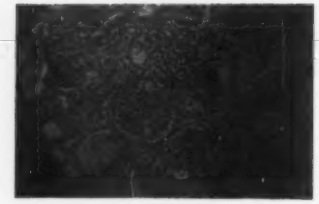


Figure 2

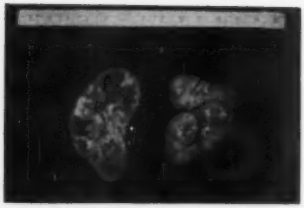
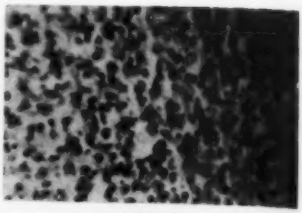


Figure 3

Figure 4



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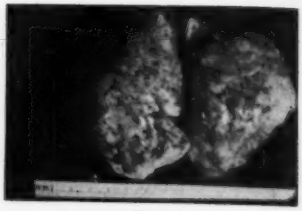

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Figure 8

Figure 9

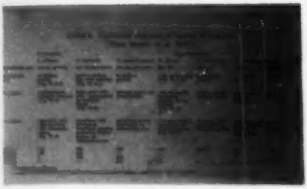

Figure 10

Figure 11

Figure 12

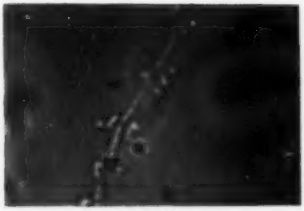

Figure 13

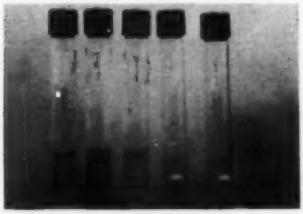

Figure 14

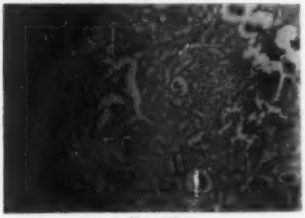

Figure 15

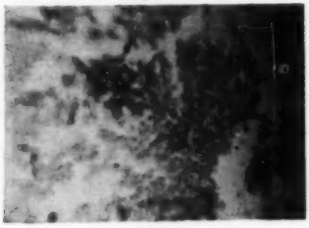

Figure 16

placed on the microscope stage.

Differential carbohydrate broths containing an indicator are inoculated and incubated at 37° for ten days (Fig. 14). C. albicans does not grow on the surface of the Sabouraud's broth as do other species of C. albicans, but is seen as a granular growth on the bottom of the tube. C. albicans produces acid and gas in glucose and maltose, acid in sucrose and no change in lactose broth. These tests are confirmatory for the presence of C. albicans; however, a pathogenicity test must be performed before any significance can be attached to their presence. We make this statement because of the fact that ten to thirty per cent of normal individuals will have saprophytic strains of monilia in their sputum. One ml. of a 1% suspension of C. albicans introduced into the ear vein of a rabbit results in death in four days. At autopsy the kidneys are swollen (Fig. 15) and multitudes of light-colored, slightly raised abscesses will be demonstrated in a similar manner to the kidney shown in Case No. 5. Periodic acid stained sections of the kidney (Fig. 16) will disclose brilliantly staining fungus cells throughout the abscesses.

Summary

In summary we should like to repeat that increasing numbers of cases of pulmonary moniliasis are being reported. A further increase may result from the alteration of the intestinal flora with the use of broad-spectrum antibiotics. It is therefore necessary for all technologists to be on the lookout for monilia in the sputum in cases of lung disease and to be capable of identifying strains of C. albicans. We have presented cases illustrating pulmonary moniliasis as a primary disease entity, as a super-infection, and probably as a complication of the prolonged use of antibiotics. The mycological diagnosis has been discussed in detail.

ACKNOWLEDGMENT

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3. Moss, E. S. and McQuown, A. L.: Atlas of Clinical Mycology, Williams and Wilkins Co., 1953, pp 50-58.

ABSTRACTS

ENTERIC CYTOPATHOGENIC HUMAN ORPHAN (ECHO) VIRUSES

Committee on the ECHO Viruses, (National Foundation for Infantile Paralysis), Science, 122, 1187-8 (1955).

Enteric cytopathogenic viruses were obtained from patients with aseptic meningitis syndrome (often diagnosed as non-paralytic poliomyelitis) and from healthy children in different parts of the world.

Viruses of multiple antigenic types are indicated from preliminary studies. A group of investigators—the Committee on the ECHO Viruses—have exchanged individual prototype strains and serums for study of cross-neutralization tests by a uniform adopted technique.

Thirteen antigenically distinct viruses were differentiated. Some of these viruses were formerly known in previous literature as "orphan viruses" and others as "human enteric viruses" are now classified as the "enteric cytopathogenic human orphan (ECHO) group."

The committee has presented their antigenic classification as a preliminary step in understanding the part such viruses found in the human enteric tract have in disease.

SURVIVAL OF BACTERIA UPON REPEATED FREEZING AND THAWING

Arthur P. Harrison, Jr., (Vanderbilt University), J. Bact. 70, 711-5 (1955).

Lactobacillus fermenti strain 69 L-3, Escherichia coli strain 69 L-15, and Serratia marcescens strain ATCC 274 were studied to determine the effect of length of storage between successive freezing, initial cell concentration, suspending medium, and the effect of aeration.

With Lactobacillus fermenti the initial freezing-thawing showed a survival which was constant and independent of the initial cell concentration, but after a series of repeated freezings and thawings the percentage survival varied in proportion to the initial cell concentration. When logarithms of the survivors were plotted against the number of successive freezings a sigmoidal curve was obtained which was influenced by the length of the storage interval, the menstruum, the initial cell concentration, and the conditions under which the test organism was cultivated. Escherichia coli and Serratia marcescens also showed a sigmoidal curve under similar conditions but showed a straight line when the storage interval was shortened.

RAPID METHOD FOR CULTIVATION OF ACID-FAST BACILLI

Ling Sun Chu, (New York State University College of Medicine), Science 122, 1189-90 (1955).

Fourteen sterile, standard glass slide preparations were made of sediments obtained from a modified sodium hydroxide-hydrochloric acid concentration procedure. The slides were placed in sterile, standard, horizontal glass staining dishes and covered with a specially prepared culture medium and incubated at 37.5° C. Each day for fourteen days a slide was removed and stained by the Ziehl-Neelsen technique. If each of the fourteen slides showed no acid-fast bacilli the test was considered negative. Known positive specimens were used as controls in another similar staining dish.

The average number of days for growth of acid-fast bacilli was 2.5 days with the specially prepared medium while an average of 31.1 days was required for detection of growth in American Trudeau Society medium.

Two concentrations of medium were used. Both were made with lecithin (in alcohol), $Na_2HPO_4 \cdot 12\ H_2O$, KH_2PO_4, $MgSO_4 \cdot 7\ H_2O$, sodium citrate, NH_4Cl, iron ammonium citrate, asparagine, glucose, Tween 80 (10% solution), human plasma, blood-water (50%) and penicillin. The stronger concentration was used with equal amounts of the sodium hydroxide-hydrochloric acid prepared specimen and incubated for 24 hours at 37.5° C. After centrifuging the sediment was used as indicated above.

TELLURITE-GLYCINE AGAR: A SELECTIVE PLATING MEDIUM FOR THE QUANTITATIVE DETECTION OF COAGULASE-POSITIVE STAPHYLOCOCCI

Eugene Zebovitz, James B. Evans, and C. F. Niven, Jr., (University of Chicago), J. Bact. 70, 686-90 (1955).

A selective medium (tellurite-glycine agar) is introduced which shows advantages superior to other currently used media for the quantitative detection of coagulase-positive staphylococci. Black colonies are produced within 24 hours at 37° C. Detection of coagulase-positive staphylococci has been made using this media with food products, fecal samples, soil, air, dust, and on the skin and mucous membranes. The medium contains tryptone, yeast extract, mannitol, K_2HPO_4, lithium chloride, glycine, agar and potassium tellurite (added aseptically after sterilization). The pH is adjusted to 7.2 before autoclaving.

BLOOD PYRUVIC ACID, SERUM GAMMA GLOBULIN AND OTHER TESTS OF HEPATIC FUNCTION

Raj K. Parida and Robert M. Kark, (Cook County Hospital, Chicago), J. Lab. Clin. Med. 47, 42-50 (1956).

Thirty-nine patients were examined for the basal blood pyruvate levels and these results compared with seven tests of hepatic function. Two-thirds of twenty-four patients ill with hepatic disease and four of fifteen patients without liver disease had high levels showing a limited value to the determination of pyruvate blood levels in the detection of hepatic dysfunction in man. Positive correlation was found between concentrations of serum gamma globulin and the basal levels of blood

pyruvate. Thymol turbidity, which depends on the serum concentration of gamma globulin, also showed a similar correlation. A negative correlation was found with serum cholinesterase activity.

PELGER-HUET ANOMALY OF THE LEUKOCYTES

Arthur Klein, Allen E. Hussar, and Siegbert Bornstein, (Franklin Delano Roosevelt Veterans Administration Hospital), New Eng. J. Med. 253, 1057-61 (1955).
The literature is reviewed along with a report on a case of heterozygous Pelger-Huet anomaly. The problem of the anomaly and the practical importance of its recognition in persons affected is discussed. A study of a stained blood smear in the Pelger-Huet anomaly shows the granulocytes to have the following deviations from normal: the nuclear-cytoplasmic ratio was noted to be smaller than normal. The number of stab forms was greatly increased. Hardly any polymorphic nuclei with more than 2 segments were seen; the nuclei were of peanut, pince-nez, or dumb-bell shape. All granulocytic nuclei were coarse, mature, and pitted with condensed masses of chromatin. Bone marrow examination shows an increase in myelocytes, meta-myelocytes and band forms and a lack of highly segmented granulocytes. The cell nuclei of the myelocytes are dark and coarsely pitted, instead of the more typical, lighter color of the younger cells. The chromatin tends to clump in the center of the nucleus, which is frequently at the outer periphery of the cell, and the nucleus is relatively small in comparison to the cytoplasm. From the standpoint of pathology, the Pelger-Huet anomaly is apparently a completely harmless condition.

The Pelger-Huet anomaly has been demonstrated to be a hereditary nonsex-linked simple mendelian-dominant characteristic. The authors suggest that the demonstration of the anomaly may eventually have some medicolegal application for proof of personal identity or in paternity proceedings.

THE CLINICAL SIGNIFICANCE OF ERYTHROCYTE CHOLINESTERASE TITERS I. A METHOD SUITABLE FOR ROUTINE CLINICAL USE, AND THE DISTRIBUTION OF NORMAL VALUES.

Jean Captain Sabine, (Health Div., Los Alamos Scientific Laboratory of the Univ. of Cal.), Blood 10, 1132-8 (1955).
In this paper the procedure for determining cholinesterase titer of erythrocytes is given in detail with notes on reagents and equipment. The procedure can be carried out by a competent technician and one person can complete the determinations on six samples in about two hours and twelve in about three hours. The amount of blood required can be obtained from the finger if a micro or semi-micro hematocrit apparatus is available. The colorimetric method of Hestrin for the determination of acetylcholine and other carboxylic acid derivatives, based on the reaction of acetylcholine with hydroxylamine to form acethydroxamic acid which forms a colored complex with ferric ions, has been adapted for cholinesterase determinations on blood. Any instrument can be used which gives a good straight line relation between concentrations of acetycholine and optical density. Either the Coleman Junior Spectrophotometer or other suitable instrument.

Evidence has been published that the cholinesterase titer of erythrocytes in the peripheral blood is an indication of the activity of the bone marrow. Additional data, to be published in Part II of this paper, will further indicate that under many circumstances the cholinesterase titer to be more informative than the reticulocyte count.

GENETICS OF SICKLING

(Editorial), Lancet, 2; 1073-5 (1955).
This editorial raises two questions. (1) How does a gene which in homozygous form produces a disease that is generally fatal in childhood or adolescence, attain such frequency in certain populations that up to 40% of the people may be heterozygous for it? (2) How is it that such a gene is very common in some populations and virtually absent in others? If homozygous individuals die young, each successive generation should show a steady decline in the frequency of the gene. Dr. J. V. Neel of the U. of Mich. pointed out two possible explanations. First, fresh mutations from the normal to the abnormal genes lost in each generation by the relatively early death and consequent infertility of the abnormal homozygotes. Alternatively, the heterozygotes may have a higher effective fertility than the normal homozygotes ("balanced polymorphism") because, on the average, more homozygotes survive to adult life than do normal homozygotes. The editor suggest studies be made in different areas throughout the world.

FEVER AND HEAT AS CAUSE OF FRAGMENTATION OF RED CELLS IN PATIENTS WITH MEDITERRANEAN ANEMIA

L. Perosa and M. Dibenedetto dell'Aquila, (Medical University Clinic, Bari), Acta Haemat. 14, 209-12 (1955).
In five patients an experimentally induced rise of temperature showed a production of hemolytic crises in patients affected by Mediterranean anemia, and that the red cells of those patients, in vitro were more sensitive to heat than normal red cells. The results showed that each fever attack is followed by an accentuation of the hemolytic picture and increased bilirubinimia and hemolytic index. The authors have some excellent micro photographs showing the red cells before and after the induced rise of temperature in the five patients.

BROADCASTING FROM "THE PARTY LINE"

By Doris S. Whitney, MT (ASCP)

!Ready with your Person to Persons Call!

Did you read the February 18th issue of the SATURDAY EVENING POST? Quebec City was featured as its 17th in the "Cities of the World Series." The author takes you to "the most glamorous European City this side of the Atlantic" with fascinating pictures and stories. Wasn't that timely for all of us?

Quebec Conference: June 17-22, Chateau Frontenac—"Service through Cooperation."

ASMT is twenty-four years old and is crossing the border for the first time to share its convention with that of Canada's. This is an expreience in our growth that we should cherish—like a child's mark of growth on the kitchen wall.

Conference High Light: Symposium on Enteric Diseases.

Participants in this will come from Chamblee and Toronto and will include Dr. Fournelle from Alaska.

Keynote Speaker: The keynote speaker as well as a participant in the Symposium will be the THE authority on enteric diseases, Dr. F. Kauffmann of Copenhagen, who has accepted the invitation extended by Lederle to come here expressly for this purpose.

There is simply "reams" to tell you about the scientific program, the refresher courses at Laval, one of the oldest universities of learning in North America, and the entertainment which includes teas, a very special luncheon where the provinces will be sponsors (I **so** hope I draw the Nova Scotia table) and climaxed by the BUFFET (wait 'til you hear more about this). But this time let's chat about the trip itself.

The February ASMT NEWS RELEASE gave you an inkling as to how to travel—on wings, Canadian Pacific Railway or bus. (The "thumb" was thought infeasible because of our "position" in life). You know, Cartan Travel Bureau for one is rather perturbed at our societies. When I called on them for travel ideas one agent said, "Are YOU the ones that have tied up the Chateau Frontenac and the Saguenay River trip for a week in June!" Wonder what type of doghouse the travel bureaus have reserved for us.

If you possibly can, take extra time to travel on the way or after the conference. You will be in a land of charm that beckons you first to the river to watch the ocean-going liners or fishing boats, then to the quaint city streets, on again through those historic shrines and cathedrals, the beautiful Montmerency Falls and then to catch the changing of the guards.

An overnite Saguenay River trip will give you a comprehensive view of the beautiful country and towns as you sail down the majestic St. Lawrence past Murray Bay, St. Simeon, the high rock bluffs of Cape Eternity and Cape Trinity as you enter the Saguenay River. There is a stop for sightseeing at Tadoussac, traditional meeting place for centuries of Indians from Hudson Bay to Florida.

If you drive—opportunity is limitless—the Gaspé Peninsula is another world, Prince Edward Island, Cape Breton Island, Nova Scotia, a car ferry from there to Bar Harbor, down the coast of Maine and back home! (My thumb won't want to cooperate with any dignity I may possess.)

How about a new ASMT membership slogan—with apologies to the Navy?

!JOIN THE ASMT AND SEE THE WORLD! Or NORTH AMERICA AT LEAST!

Ed. Note: The "Party Line" is usually a "hook-up" and medium for an exchange of ideas for Public Relations Chairmen and presidents of the constituents societies of ASMT. With Mrs. Whitney's permission we are making this a broadcast to all the readers of the AJMT.

Ample time will be allowed to visit the exhibits—

MAKE YOUR RESERVATION TODAY

for the FIRST NORTH AMERICAN CONFERENCE OF MEDICAL LABORATORY TECHNOLOGISTS

June 17-22, 1956

Theme: Service Through Cooperation

There will be a SYMPOSIUM on Enteric Diseases with special emphasis upon techniques for diagnoses in cold climates, with F. KAUFFMANN, M.D., Chief International Salmonella Center, State Serum Institute, Copenhagen, Denmark, and H. J. FOURNELLE, Ph.D., Chief Department of Bacteriology, Arctic Health Research Laboratory, USPHS, Anchorage, Alaska, as two members of the panel.

CONFERENCE DEMONSTRATIONS will be on Public Relations and Recruitment and Hospital Accreditation (Canadian).

There will be ten REFRESHER COURSES:

BIOCHEMISTRY (2 courses)
1. Biochemistry—general
2. Spectrophotometry

HEMATOLOGY (2 courses)
1. General course
2. Sternal Marrow

BACTERIOLOGY (1 course)

SEROLOGY (1 course)

HISTOLOGY (1 Course)

BLOOD BANKING (1 course)

AND TWO REFRESHER COURSES CONDUCTED IN FRENCH, on general fundamentals of laboratory techniques.

See your January-February Journal for detailed description.

Please note your first, second, and third choice. These will run simultaneously so one person can take only one course.

Each INSTRUMENTATION DEMONSTRATION will accommodate 25 persons and will last for one hour. Sign up for one in advance to declare your intention, and as the schedule is developed at convention time, you may register for additional periods if you wish.

Papers on every phase of medical technology will be presented by American and Canadian Society members, as well as by guest speakers. To add to the international spirit, there will be papers by Miss Elisabeth Pletscher, of Zurich, Switzerland, who will read hers in both English and French, and by Mr. Andrew Fraser, of Edinburgh, Scotland.

The opening ceremonies will be at 3:00 P.M. on Sunday, followed by a tea.

The buffet dinner will be on Wednesday evening. The tea on Thursday afternoon will mark the close of all but the final business sessions.

PLAN TO ATTEND ! !

The First North American Conference of Medical Laboratory Technologists is an event of great importance to each of us. The American Society of Medical Technologists and the Canadian Society of Laboratory Technologists will here combine their Annual Conventions for the first time, and a very large attendance is anticipated. The Conference site is the historic city of Quebec. Quebec City is one of the oldest cities in Canada with many FIRSTS to its credit. Laval University where our refresher courses will be held was started as a Seminary in 1668. This was the beginning of one of the oldest Universities in Canada and on the North American Continent.

ACCOMMODATIONS:

The Conference headquarters will be the Chateau Frontenac Hotel where accommodation can be reserved by completing and returning the card which was enclosed with your January-February journal. If you wish accommodation elsewhere, see list of other hotels and motels.

Make HOTEL RESERVATIONS DIRECT TO THE HOTEL CHATEAU FRONTENAC —QUEBEC CITY, QUEBEC, CANADA—headquarters. Other hotels in the vicinity are:

Clarendon Hotel, 57 Ste. Anne Street
St. Louis Hotel, 33 St. Louis Street
Chateau Laurier Hotel, 107 Grande-Allee
Chateau Normandie Hotel, 12½ Ste. Anne Street
Victoria Hotel, 56 St. Jean Street

Motels approximately 3 to 3½ miles from the Chateau are:
Motel des Laurentides, 350 Blvd. Ste. Anne
Motel du Pont, Blvd. Wilfred Laurier
Motel Bouchard, 333 Blvd. Ste. Anne
Motel l'Abitation, 2828 Blvd. Wilfred Laurier

SISTERS' ACCOMMODATION is under the direction of Reverend Sister St. Monica, c/o Laboratory, Hospital Du St. Sacrement, Quebec City. Notices about Sister Accommodation and entertainment will be going out directly to the Sisters.

GUESTS:

Other than members of A.S.M.T. and C.S.L.T. will be welcome at all the scientific and social sessions. During the Business Meetings information will be available at the Registration desk for assistance in their entertainment.

FEES:

Registration fee:
— members $5.00
— registered students must have written verification or membership card
— non-members $6.00

All other charges are as outlined on the Advance Registration form.

HOUSE OF DELEGATES: The Annual Meeting of the ASMT House of Delegates will take place on WEDNESDAY afternoon and all day Friday and will be open to **members** only.

TICKET SALES:

ATTENTION all those NOT attending the full Convention. It is necessary to notify well in advance those catering to our eating pleasure, of numbers to be served. Tick-

ets for planned meals as outlined in the program, will not be available later than six (6) hours prior to the time as scheduled on the program.

WEATHER CONDITIONS:

The average summer temperature in Quebec seldom exceeds 75° F. It is therefore suggested that delegates bring a medium weight coat for evening wear.

TOURS:

There will be people on hand to assist in the arranging of tours throughout the city and environs, as well as Ste. Anne de Beaupre and Montmorency Falls.

ADVANCE REGISTRATION for the FIRST NORTH AMERICAN CONFERENCE of MEDICAL LABORATORY TECHNOLOGISTS, ANNUAL CONVENTIONS of the AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS and the CANADIAN SOCIETY OF LABORATORY TECHNOLOGISTS

June 17-22, 1956—Chateau Frontenac, Quebec, P.Q., Canada

Please fill in and mail with your check or money order to Miss Mary Nagai, M.T. (ASCP), 25 Hermann Professional Building, Houston 25, Texas. If you do not register for a Refresher course, your advance registration application and fees will be accepted if mailed on or before May 20, 1956.

NAME_____ DATE_____
Please Print

BUSINESS ADDRESS_____

_____
city zone state or province country

REGISTRATION FEE $5.00

No. tickets

____	Buffet & Reception	$9.00	____
____	Luncheon	$3.00	____
____	Sunday Tea	$1.75	____
____	Thursday Tea	$1.75	____
____	Instrumentation Demonstration	$ .50	____
1 (Only)	Refresher Course	$5.00	

Total

Do not write in this space

Make your check payable to the First North American Conference of Medical Laboratory Technologists

Please note: Member { ASMT ☐ CSLT ☐ } Student { ASMT ☐ CSLT ☐ } Visitor ☐

Student members of ASMT will have the Registration fee refunded unless they sign for a refresher course.

ALL REFRESHER course registrations must be mailed and PAID by April 15, 1956

REFRESHER COURSES—Please Note 1st, 2nd, and 3rd choice:

☐ Biochemistry (general)
☐ Biochemistry (spectrophotometry)
☐ Hematology (general)
☐ Hematology (sternal marrow)*
☐ Bacteriology
☐ Histology
☐ Blood Banking
☐ Serology
☐ General Procedures } Two courses conducted in French

*Limited to 30. Fill in form on page 126.

All ADVANCE REGISTRATION APPLICATIONS must be accompanied by the $5.00 REGISTRATION FEE as well as the refresher fee. Unless receipt is requested, your canceled check will serve as receipt.

BONE MARROW REFRESHER COURSE

Name_____
please print

Address_____
please print city state

Laboratory Address:_____

Department_____

Duties_____

_____

Train students: Yes:_____ No:_____

Approximate number students trained per year_____

Time in Hematology_____
months years

Academic standing:

Senior Matriculation_____
(Canadian)

Student in Approved School_____
(American)

University_____
no. of years

_____
degree

Member of the CSLT ☐
(please check)

Member of the ASMT ☐

I hold a specialist certificate_____

(If so, in what field)_____

I am interested in this course because_____

_____

_____

Further Comments:_____

_____

_____

_____

Signed_____

ARTICLES OF INCORPORATION

AND BY-LAWS

of

The American Society of Medical Technologists

A Michigan Corporation
(Non-Profit)

1953

Supplement: American Journal of Medical Technology
Vol. XXII, No. 2, March-April, 1956

ARTICLES OF INCORPORATION

OF THE

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

(NON-PROFIT)

These Articles of Incorporation are signed and acknowledged by the incorporators for the purpose of forming a non-profit corporation under the provisions of Act No. 327 of the Public Acts of 1931, known as the Michigan General Corporation Act, as follows:

ARTICLE I
Name

The name of this corporation is American Society of Medical Technologists.

ARTICLE II
Purposes

The purposes of this corporation are:

To promote higher standards in clinical laboratory methods and research.

To elevate the status of those specializing in medical laboratory technique.

To create mutual understanding and cooperation between the medical technologist and physicians and all others who are employed in the interest of individual or public health.

To issue charters to constituent societies.

To promote the mutual aid and benefits of its members.

In general to do anything and everything necessary and proper to the conduct of a society of this nature, and for the purpose of attaining or furthering any of its objects to do any and all other acts and things, and to exercise any and all other powers which now or hereafter may be authorized by law.

ARTICLE III
Location

The location of the corporation is the City of Detroit, in the County of Wayne, State of Michigan, Post Office address of registered office in Michigan is 1100 Dime Building, Detroit 26, Michigan.

ARTICLE IV
Plan

Said corporation is organized upon a non-stock basis. (Original Articles included Statement of Assets at time of incorporation; that portion is not reproduced here.) Said corporation is to be financed under the following general plan: Annual Dues.

ARTICLE V
Incorporators

The names and places of residence, or business, of each of the incorporators are as follows:

John H. Conlin, 10439 East Outer Drive, Detroit, Michigan
Christine Clayton Seguin, 8056 Lincoln Avenue, Niles Center, Illinois
Robert C. Jenkins, 948 Edgecomb Place, Chicago, Illinois

ARTICLE VI
First Board of Directors

The names and addresses of the first board of directors (or trustees) are as follows:

Sarah McCarty, 805 Medical Arts Building, Birmingham, Alabama
Robert Jenkins, 948 Edgecomb Place, Chicago, Illinois
John H. Conlin, 10439 East Outer Drive, Detroit, Michigan

ARTICLE VII
Term of Incorporation

The term of this corporation is perpetual, subject to the right of the Legislature to amend Section 12, Act 327, Public Acts, 1931.

ARTICLE VIII
Membership

The membership of this Society shall consist of such classes and subclasses as may be provided by the By-Laws of this Society, and the members in each of such classes and subclasses shall have such rights and privileges and shall be governed by such rules of eligibility, admission, retention and dismissal as may be prescribed by the By-Laws.

ARTICLE IX
Officers, Directors, Advisory Council

Section 1. The officers of this Society shall be: a President, a President-Elect, a Recording Secretary, and a Treasurer, and such other officers as may be provided by the By-Laws of this Society. The respective terms of office and duties of all of such officers shall be as prescribed by the By-Laws.

Section 2. The Board of Directors of this Society shall consist of the President, the immediate past President, the President-Elect, the Recording Secretary and the Treasurer, during their respective terms of office, and in addition six members of the Society to be elected by the House of Delegates. The Board of Directors shall constitute the governing body of this Society while the House of Delegates is not in session.

Section 3. There shall also be an Advisory Council whose membership shall consist of the members of the Board of Directors of this Society and two representatives from each constituent society as may be provided for by the By-Laws of this Society. The Advisory Council shall consider and

promote the interests of this Society and shall perform such other duties as may be provided by the By-Laws.

Section 4. The Executive Secretary shall be included as a non-voting member of the Board of Directors and of the Advisory Council.

ARTICLE X
House of Delegates

Section 1. There shall be a House of Delegates whose membership shall consist of: (a) the members of the Board of Directors and the members of the Advisory Council or the respective alternates for any members of the Advisory Council; and (b) delegates (or their alternates, as the By-Laws may provide) elected or designated by the constituent societies on a representative basis as provided for in the By-Laws of this Society.

Section 2. The duties of the House of Delegates shall be those of the governing body of this Society.

ARTICLE XI
Constituent Societies

Section 1. This Society shall be composed of the constituent societies which now hold, and which may hereafter be granted, charters from this Society. Each state, the District of Columbia and each territory, possession or dependency of the United States of America, and each foreign nation shall constitute a geographical unit for the chartering of a constituent society therein.

Section 2. Each society which is now chartered by this Society as a subordinate society shall be duly recognized as a constituent society, and additional constituent societies may be formed and chartered in accordance with such requirements and procedure as may be provided by the By-Laws of this Society.

Section 3. The charter of any constituent society may be revoked or reinstated upon such grounds and conditions and in accordance with such procedure as may be prescribed by the By-Laws of this Society.

ARTICLE XII
By-Laws

The House of Delegates shall have the power to adopt, enact and amend By-Laws consistent with the Articles of Incorporation and the general objects and purposes of the Society and not in conflict with the laws of the State of Michigan.

ARTICLE XIII
Amendments

These Articles of Incorporation may be amended by a majority vote of the members of this Society entitled to voting privileges by a vote in such manner as the By-Laws shall provide, provided that notice of the proposed amendment or amendments shall have been published in the Society's official organ at least sixty days and not more than one hundred twenty days in advance of the annual or special meeting at which such proposed amendment or amendments shall be submitted or shall have been mailed to the members of the Society having voting privileges at least sixty days and not more than one hundred twenty days prior to such meeting, and that the proposed amendment or amendments shall have been approved by a two-thirds vote of the Board of Directors within one year prior to such meeting.